



Two New Eudesmane Sesquiterpenoids from the Flowers of *Chrysanthemum indicum*

Jun-Li Yang¹ · Lei-Lei Liu¹ · Yan-Ping Shi¹

Received: 4 January 2019 / Accepted: 20 February 2019 / Published online: 28 February 2019
© The Author(s) 2019

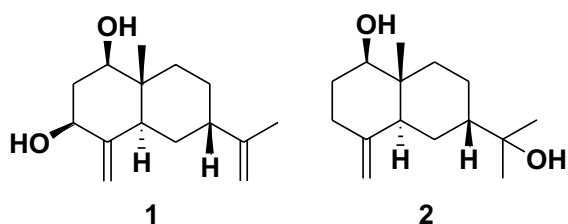
Abstract

The flowers of *Chrysanthemum indicum*, i.e. Ye-ju-hua recorded in the Chinese Pharmacopoeia, has been widely used in China as an important heat-clearing and detoxifying herb for the treatment of inflammation, headache, and vertigo. A phytochemical investigation of this herb has led to the isolation of two new eudesmane sesquiterpenoids, 7-*epi*-eudesm-4(15),11(13)-diene-1 β ,3 β -diol (**1**) and 7-*epi*-1 β -hydroxy- β -eudesmol (**2**). The molecular structures of these new sesquiterpenoids were established based on the comprehensive spectroscopic analyses, including NMR, MS, and IR, and comparing with the literatures.

Graphical Abstract



Flowers of *Chrysanthemum indicum*
(野菊花)



Keywords *Chrysanthemum indicum* · Sesquiterpenoids · Eudesmane

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13659-019-0199-9>) contains supplementary material, which is available to authorized users.

✉ Yan-Ping Shi
shiyp@licp.cas.cn

¹ CAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

1 Introduction

The plants of Asteraceae family, which contains 25 000–30 000 species belonging to over 1000 genera, have been considered as rich sources of eudesmane sesquiterpenoids [1]. Till now there have been over 1000 naturally occurring eudesmane sesquiterpenoids identified from the Asteraceae family, and these plant metabolites possessed diverse oxygenation and cleavage patterns [2]. Eudesmane-type sesquiterpenoids exhibit a wide range of biological activities, such as plant growth regulating, insect antifeedant, antifungal, anti-tumour, antibacterial, and antiviral activities [3–11]. Due to these, eudesmane-type sesquiterpenoids

have long been the research focus of the phytochemists and pharmacologists.

The flowers of *Chrysanthemum indicum* has long been used a traditional heat-clearing and detoxifying herb in China. It is called Ye-ju-hua in the Chinese Pharmacopoeia [12]. The main usage of this traditional Chinese medicine was to treat inflammation, headache, and vertigo and for the preparation of a bitter tea used for antibacterial, antioxidant, and anti-inflammatory purposes [13]. It has long been our goal to find more novel sesquiterpenoids from traditional Chinese herbs [14–18]. As part of this program, an investigation of an aqueous EtOH extract of the flowers of *C. indicum* afforded 2 further new sesquiterpenoids (Fig. 1), 7-*epi*-eudesm-4(15),11(13)-diene-1 β ,3 β -diol (**1**) and 7-*epi*-1 β -hydroxy- β -eudesmol (**2**). In this paper we will report the procedures for the isolation and structure determination of the two new isolates.

2 Results and Discussion

Sesquiterpenoid **1** was isolated as a white solid with a optical rotation value of $[\alpha]_D^{20} + 5.0$ (c 0.2, acetone), which suggested this compound could be a racemate. It was assigned a molecular formula of $C_{15}H_{24}O_2$ as determined based on an ion peak at m/z 219.1750 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{23}O$, 219.1743) in its positive HRESIMS, which suggested four degrees of unsaturation. The IR absorptions at 3384 and 1654 cm^{-1} suggested the presence of hydroxy and olefinic functionalities, respectively. The 1H and ^{13}C NMR data of **1** revealed (Table 1) an angular methyl (δ_H 0.74, δ_C 9.9), a vinyl methyl (δ_H 1.70, δ_C 22.2), two oxymethines (δ_H 3.44, δ_C 77.1; δ_H 4.06, δ_C 70.4), and two terminal double bonds (δ_H 4.73, 5.11, δ_C 103.4, 151.6; δ_H 4.79, 4.91, δ_C 110.9, 146.4). These observations suggested that **1** possessed a eudesmane framework with two hydroxy groups and two terminal double bonds [14–18]. From the HMBC experiment (Fig. 2), the angular methyl (H_3 -14) correlated with C-1, C-5, C-9 and C-10 (δ_C 77.1, 38.9, 32.3 and 40.5 respectively), indicating that one hydroxyl group was linked at C-1. Similarly, the exomethylene protons (H_2 -15) correlated with C-3 and C-5 (δ_C 70.4 and 38.9), which clearly indicated that another hydroxyl group was located at C-3. In the NOE difference experiment, irradiation of H-1 (δ_H 3.44) enhanced the resonances of H-3 (0.81%), H-2 α (1.47%), and H-5 (1.15%), but not H_3 -14, indicating that H-1, H-3 and H-5 were all α -oriented (assuming that H_3 -14 was β -oriented). Comparison of the carbon signal at C-7 (δ_C 38.4) with that of ligucyperonol (δ_C 45.1) [19] and β -dictyopterol (δ_C 45.3) [20], suggested a β -orientation of H-7. Finally sesquiterpenoid **1** was elucidated as 7-*epi*-eudesm-4(15),11(13)-diene-1 β ,3 β -diol.

Table 1 NMR Spectroscopic Data (in $CDCl_3$, 400 MHz) of Sesquiterpenoids **1** and **2**

No.	1		2	
	1H (δ)	^{13}C (δ)	1H (δ)	^{13}C (δ)
1	3.44 dd (12.0, 4.4)	77.1 (d)	3.45 dd (11.6, 4.4)	79.3 (d)
2	2.23 m, 1.51 m	40.8 (t)	1.82 m, 1.51 m	31.4 (t)
3	4.06 dd (12.0, 5.2)	70.4 (d)	2.32 m, 2.17 m	34.3 (t)
4		151.6 (s)		149.3 (s)
5	1.73 m	38.9 (d)	2.17 m	41.8 (d)
6	1.85 m, 1.67 m	25.1 (t)	1.73 m, 1.63 m	23.5 (t)
7	2.45 brs	38.4 (d)	1.68 m	41.6 (d)
8	1.88 m, 1.67 m	22.9 (t)	1.78 m, 1.63 m	20.8 (t)
9	1.63 m, 1.37 m	32.3 (t)	1.62 m	34.0 (t)
10		40.5 (s)		39.6 (s)
11		146.4 (s)		74.4 (s)
12	1.70 s	22.2 (q)	1.27 s	29.6 (q)
13	4.91 d (1.6) 4.79 s	110.9 (t)	1.26 s	29.2 (q)
14	0.74 s	9.9 (q)	0.73 s	11.1 (q)
15	5.11 s, 4.73 d (1.6)	103.4 (t)	4.78 d (1.2), 4.54 s	106.8 (t)

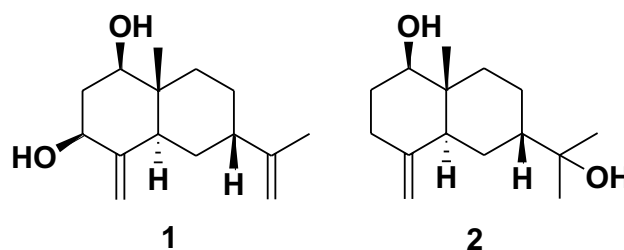


Fig. 1 Molecular structures of sesquiterpenoids **1** and **2**

Sesquiterpenoid **2** was obtained as a white solid with $[\alpha]_D^{20} + 3.0$ (c 0.35, acetone), which indicated it maybe exist as a racemate. Its molecular formula was assigned as $C_{15}H_{26}O_2$ on the basis of an ion at m/z 261.1815 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1825) in the positive HRESIMS. Its IR absorptions at 3323 and 1645 cm^{-1} indicated the occurrence of hydroxy and olefinic functionalities, respectively. The 1H and ^{13}C NMR data of **2** demonstrated three methyls (δ_H 1.11, 1.22, 1.32; δ_C 11.1, 29.2, 29.6), one oxymethine (δ_H 3.45; δ_C 79.3), one oxygenated tertiary carbon (δ_C 74.4), and one terminal double bond (δ_H 4.54, 4.78; δ_C 106.8, 149.3). These observations indicated **2** as an eudesmane sesquiterpenoid possessing two hydroxy groups and one terminal double bond [14–18]. Furthermore, its planer structure was constructed via HMBC experiment as shown in Fig. 2. From the NOE difference experiment, irradiation of H-1 (δ_H 3.45)

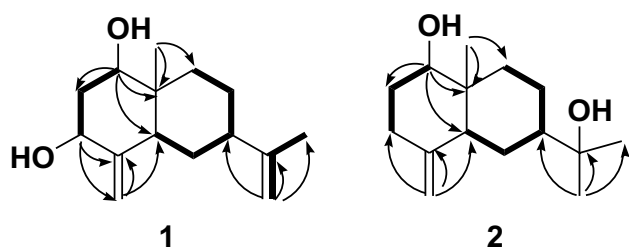


Fig. 2 Key COSY (H \rightarrow H), HMBC (H \rightarrow C) correlations of **1** and **2**

enhanced the signals of H-3 α (1.47%) and H-5 (1.11%) indicating that H-1 and H-5 was α -oriented. The chemical shifts of C-1/2/10 of compound **8** (δ_C 79.3/31.4/39.6 in CDCl₃), which were similar to those of β -dictyoptero (δ_C 79.3/31.5/40.2 in CDCl₃) [20], were used to determine the β -oriented Me-14. The β -orientation of H-7 was deduced from dramatic differences in chemical shifts of C-5/7/9 in compound **8** (δ_C 41.8/41.6/34.0 in CDCl₃) and 1 β -hydroxy- β -eudesmol (δ_C 47.5/48.9/36.9 in CDCl₃) [21]. Thus, sesquiterpenoid **2** was established as 7-*epi*-1 β -hydroxy- β -eudesmol.

3 Experimental Section

3.1 General

Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 1 dm cell. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer. Chemical shifts are given as δ (ppm) using TMS as internal standard. HRESIMS were carried out on a Bruker APEX II mass spectrometer. Sephadex LH-20 (GE Healthcare Bio-science AB, Sweden), C₁₈ reversed-phase silica gel (YMC, ODS-A, AA120S50), Silica gel (200–300 mesh, Qingdao Marine Chemical factory, Qingdao, China.) were used for column chromatography (CC). TLC using silica GF₂₅₄ (10–40 μ m) was detected at 254 nm and spots were visualized by spraying with 5% H₂SO₄ in EtOH (v/v) followed by heating.

3.2 Plant Material

The flowers of *C. indicum* was purchased from Huanghe Medicinal Material Market in Gansu in 2009 and identified by Associate Prof. H.Y. Qi of CAS Key Laboratory of Chemistry of Northwestern Plant Resources in LICP, where a voucher specimen (No. ZY2009C001) was deposited.

3.3 Extraction and Isolation

The air-dried flowers of *C. indicum* (2.0 kg) were pulverized and extracted with 95% EtOH (3 \times 5 L) at 50 $^{\circ}$ C for 3 h each time. The combined extracts were evaporated to dryness under reduced pressure. The resulting residue was mixed with H₂O (1.5 L) to form a suspension and partitioned successively with petroleum ether (PE), EtOAc, and *n*-BuOH. The PE-soluble part (60 g) was subjected to silica gel CC with a gradient system of PE–EtOAc (60:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1 and 1:1) to afford eight fractions (A–H) based on TLC analysis. Fractions F–G was purified over a column of Sephadex LH-20 using CHCl₃–MeOH (2:1) to give the sub-fractions (F1 and G1) without pigments. Sub-fraction F1 was separated by silica gel CC eluted repeatedly with CHCl₃–EtOAc (25:1, 15:1 and 10:1), PE–EtOAc (10:1), CHCl₃–Me₂CO (15:1), and a column of C₁₈ reversed-phase silica gel (MeOH–H₂O, 70:30, 60:40), to give **2** (12.3 mg). Sub-fraction G1A was repeated purified by silica gel eluted with CHCl₃–Me₂CO (10:1) and PE–Me₂CO (8:1.5), and then over a C₁₈ reversed-phase silica gel eluted by (MeOH–H₂O, 60:40, 50:50) to yield **1** (16.1 mg).

3.4 Characteristic Data of Compounds

7-*epi*-eudesm-4(15),11(13)-diene-1 β ,3 β -diol (**1**): White solid; $[\alpha]_D^{20} + 5.0$ (*c* 0.2, acetone); IR (KBr) ν_{\max} : 3384, 2924, 1654, 1458, 1064, 803 cm⁻¹; ¹H and ¹³C NMR data, Table 1; HRESIMS: *m/z* 219.1750 [M – H₂O + H]⁺ (calcd for C₁₅H₂₃O, 219.1743).

7-*epi*-1 β -hydroxy- β -eudesmol (**2**): White solid; $[\alpha]_D^{20} + 3.0$ (*c* 0.35, acetone); IR (KBr) ν_{\max} : 3323, 2970, 1645, 1455, 1034, 884 cm⁻¹; ¹H and ¹³C NMR data, Table 1; HRESIMS: *m/z* 261.1815 [M + Na]⁺ (calcd for C₁₅H₂₆O₂Na, 261.1825).

Acknowledgements This work was financially supported by National Natural Science Foundation of China (Nos. 81673325, 81711540311, and 21705156), the International Partnership Program of CAS (No. 153631KYSB20160004), Gansu Province Key International S&T Cooperation Project (No. 18YF1WA127), and CAS Pioneer Hundred Talents Program.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing financial interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. L.-L. Liu, T.K.Q. Ha, W. Ha, W.K. Oh, J.-L. Yang, Y.-P. Shi, J. Nat. Prod. **80**, 298–307 (2017)
2. Q.X. Wu, Y.P. Shi, Z.J. Jia, Nat. Prod. Rep. **23**, 699–734 (2006)
3. B.M. Fraga, Nat. Prod. Rep. **30**, 1226–1264 (2013)
4. B.M. Fraga, Nat. Prod. Rep. **29**, 1334–1366 (2012)
5. B.M. Fraga, Nat. Prod. Rep. **28**, 1580–1610 (2011)
6. B.M. Fraga, Nat. Prod. Rep. **27**, 1681–1708 (2010)
7. B.M. Fraga, Nat. Prod. Rep. **26**, 1125–1155 (2009)
8. B.M. Fraga, Nat. Prod. Rep. **25**, 1180–1209 (2008)
9. B.M. Fraga, Nat. Prod. Rep. **24**, 1350–1381 (2007)
10. B.M. Fraga, Nat. Prod. Rep. **23**, 943–972 (2006)
11. B.M. Fraga, Nat. Prod. Rep. **22**, 465–486 (2005)
12. Chinese Pharmacopoeia Commission, *Chinese Pharmacopoeia* (China Medical Science Press, Beijing, 2015), p. 33
13. Z.M. Feng, S. Song, P.F. Xia, J.S. Jiang, P.C. Zhang, Helv. Chim. Acta **92**, 1823–1828 (2009)
14. H.L. Jiang, W. Ha, J.L. Yang, Y.P. Shi, Phytochemistry Lett **17**, 1–5 (2016)
15. J.L. Yang, Y.M. Zhao, Y.P. Shi, Nat. Prod. Bioprospect. **6**, 211–216 (2016)
16. X.Y. Xie, R. Wang, Y.P. Shi, Planta Med. **78**, 1010–1014 (2012)
17. J.L. Yang, Y.P. Shi, Phytochemistry **76**, 124–132 (2012)
18. J.L. Yang, R. Wang, L.L. Liu, Y.P. Shi, Planta Med. **77**, 362–367 (2011)
19. K. Naya, T. Okayama, M. Fujiwara, M. Nakata, T. Ohtsuka, S. Kurio, Bull. Chem. Soc. Jpn **63**, 2239–2245 (1990)
20. L.H. Hu, Z.L. Chen, Phytochemistry **44**, 1287–1290 (1997)
21. H.I. El-Askary, M.R. Meselhy, A.M. Galal, Molecules **8**, 670–677 (2003)