Published in final edited form as:

Tetrahedron Lett. 2015 May 20; 56(21): 2732–2734. doi:10.1016/j.tetlet.2015.04.023.

Chakyunglupulins A and B, two novel 4,8,8-trimethylcyclooct-2-enone derivatives from *Barleria lupulina*

Ki Hyun Kim^a, Jon Clardy^b, Donald Senger^c, and Shugeng Cao^{d,*}

^aSchool of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do, 440-746, Republic of Korea

^bDepartment of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, USA

^cDepartment of Pathology and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, Massachusetts 02215, USA

^dNatural Products and Experimental Therapeutics, Cancer Center, University of Hawaii, 701 Ilalo Street, Honolulu, Hawaii 96813, USA

Abstract

Two novel 4,8,8-trimethylcyclooct-2-enone derivatives, chakyunglupulins A and B, together with six known lignans were isolated from the aerial part of *Barleria lupulina*. The structures of new compounds were established by extensive spectroscopic data and HR-MS, and their absolute configurations were determined by a combination of NOE experiment and application of the modified Mosher's method.

Graphical abstract

Keywords

Barleria lupulina; Acanthaceae; 4; 8; 8-trimethylcyclooct-2-enone; chakyunglupulins A and B; lignan

Barleria lupulina Lindl. (Acanthaceae) is a medicinal plant, distributed in Southeastern Asia. This plant has been used in folk medicine for mental stress, diabetes, rheumatoid

Correspondence to: scao@cc.hawaii.edu; Tel: 808-4405208; Fax: 808-5862970.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at www.sciencedirect.com

arthritis, and snake bites.¹ Particularly, this plant is categorized as one of essential medicinal plants for primary healthcare by the Thai Ministry of Public Health, and has been used in Thai as remedies for insect bites, herpes simplex, and varicella zoster lesions.^{2,3} Recent studies indicated that *B. lupulina* extract exhibited potent antiviral activity against herpes simplex virus type 2 (HSV-2),⁴ a protective effect against experimental gastric and duodenal ulcer formation,⁵ and anti-inflammatory effects associated with reduced neutrophil migration.⁶ Previous phytochemical studies on aerial parts and leaves of this plant revealed that this plant is rich source of iridoid glucosides.^{7–12} During our ongoing search for new bioactive metabolites from medicinal plants, recently we reported four new iridoid glycosides, barlupulins A–D together with 14 known analogues from the water extracts of *B. lupulina*.¹² Our continued interest in discovering novel constituents from this plant led us to isolate two novel 4,8,8-trimethylcyclooct-2-enone derivatives, chakyunglupulins A and B, together with six known lignans. The new compounds have the unique structural skeleton of 4,8,8-trimethylcyclooct-2-enone, and the structures were established by extensive spectroscopic data, HR-MS, and a combination of NOE experiment and application of the modified Mosher's method. In this Letter, we describe the isolation and structure elucidation of chakyunglupulins A (**1**) and B (**2**) (Fig. 1)

Chakyunglupulin A (**1**) was isolated as an optically active amorphous powder, $[\alpha]_D^{25} +25.1$ (c0.10, MeOH).¹³ The molecular formula, C₁₁H₁₈O₄ of **1** was assigned from HRESI-TOF-MS indicating a pseudomolecular ion peak at m/z 197.1179 [M – H₂O + H]⁺ (calcd. for C₁₁H₁₇O₃, 197.1178). The UV spectrum displayed maximum absorption bands at 214 and 287, while the IR spectrum showed absorption bands for hydroxy (3320 cm^{–1}) and carbonyl (1651 cm^{–1}) functionalities. The ¹H NMR spectrum (Table 1) consisted of signals for one olefinic proton at δ_H 5.77 (s), an oxygenated methine proton at δ_H 4.09 (m), and three methyl protons at δ_H 1.58 (s), 1.30 (s), and 1.28 (s). The ¹H NMR spectrum also displayed two methylenes at δ_H 2.46 (ddd, J = 11.0, 3.0, 2.5 Hz); 1.41 (dd, J = 12.0, 11.0 Hz) and 2.00 (ddd, J = 12.5, 2.5, 2.0 Hz); 1.28 (overlap). The ¹³C NMR (Table 1) and HSQC spectra analysis exhibited 11 signals for 3 methyl, 2 methylene, 2 methine, and 4 quaternary carbons, which were assigned by HMBC experiments. Analysis of 2D NMR spectroscopic data allowed the gross structure of **1** to be established (Fig. 2). The fragment from C-5 to C-7 via the hydroxylated C-6 was established through the ¹H-¹H COSY and TOCSY correlations of the H-5/H-6/H-7 spin system (Fig. 2). The establishment of the α,β -unsaturated ketone moiety was determined by the HMBC correlations from H₃-9 and H₃-10 to C-1, C-7, and C-8, and from H-2 to C-1, C-3, and C-8, and by the carbon chemical shifts of C-1 (δ_C 183.6), C-2 (δ_C 113.2), and C-3 (δ_C 173.7). Finally, the HMBC correlations from H₃-11 to C-4 and C-5, and from H-2 to C-4 (Fig. 2) enabled us to finish the planar structure assignment of **1**.

The absolute configurations of **1** were determined by a combination of NOE experiment and application of the modified Mosher's method. Firstly, the absolute configuration of C-6 was established on the basis of the modified Mosher's method.^{14,15} Treatment of **1** with (*S*)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] and 4-(dimethylamino)pyridine (DMAP) in pyridine gave the (*R*)-MTPA esters **1r**. Similar treatment of **1** with (*R*)-(–)-MTPA-Cl afforded the (*S*)-MTPA esters **1s**. Analysis of the ¹H

NMR chemical shift differences (δ_{S-R}) (see Supplementary data) of the two MTPA esters allowed the assignment of the absolute configuration of C-6 as *R* (Fig. 3). In the NOESY spectrum, strong NOE correlations were observed between H-6 and H₃-10, and between H-6 and H₃-11, indicating that H-6, H₃-10 and H₃-11 were on the same side of the cyclooct-2-enone ring. Thus the absolute configuration of **1** was assigned as 4*R* and 6*R*, and structure of compound **1** was determined as shown in Figure 1.

Chakyunglupulin B (**2**) was isolated as an amorphous powder with the same molecular formula of C₁₁H₁₈O₄ to that of **1** by a pseudomolecular ion peak at m/z 197.1176 [$M - H_2O + H$]⁺ (calcd. for C₁₁H₁₇O₃, 197.1178) in HRESI-TOF-MS data.¹⁶ Comparison of the spectroscopic data (Table 1) of **2** with those of **1** revealed that the NMR data of **2** were not the same as **1**, but very similar. This implied that compound **2** is a stereoisomer of **1**. Detailed analysis of 2D NMR spectral data (¹H-¹H COSY, TOCSY, HSQC, and HMBC) of **2** led to the establishment of the planar structure of **2**, which was identical to that of **1**. The opposite value of optical rotation, $[\alpha]_D^{25} - 22.0$ (c 0.10, MeOH) of **2** to that of **1**, $[\alpha]_D^{25} + 25.1$ (c 0.10, MeOH) suggested that compound **2** has different stereochemistry from compound **1**. The absolute configuration of C-6 in **2** was determined by the modified Mosher's method,^{14,15} which proved the assignment of the absolute configuration as 6*S* (see Supplementary data). The other absolute configuration of C-4 was established as *R* by the cross-peaks of NOESY spectrum between H-6 and H₃-9, between H-5 and H-6, and between H-5 and H₃-11 as well as the absence of NOESY correlation between H-6 and H₃-11. Thus compound **2** was identified as 6-epimer of **1** as shown in Figure 1.

The known compounds were identified as buddlenol C (**3**),¹⁷ medioresinol (**4**),¹⁸ (+)-eudesmin (**5**),¹⁹ (–)-(7*R*,7'*R*,7''*R*,8*S*,8'*S*,8''*S*)-4',4''-dihydroxy-3,3',3'',5-tetramethoxy-7,9':7',9-diepoxy-4,8''-oxy-8,8'-sesquieolignan-7'',9''-diol (**6**),²⁰ (–)-(7*R*,7'*R*,7''*R*,8*S*,8'*S*,8''*S*)-4',4''-dihydroxy-3,3',3'',5',5''-hexamethoxy-7,9':7',9-diepoxy-4,8''-oxy-8,8'-sesquieolignan-7'',9''-diol (**7**),²⁰ and (+)-syringaresinol (**8**)²¹ by comparison of their physical and spectroscopic data with those previously reported in the literature.

The *in vitro* cytotoxic activity of all of the isolated compounds, except **8** (due to insufficient material being available) was evaluated against two Stat3-activated cancer cell lines: A2780cisR (cisplatin-resistant human ovarian cancer cell line), MDA-MB-231 (human breast cancer cell line) and U251 (human glioblastoma cancer cell line), and control cell line NIH3T3.¹² But they didn't show any cytotoxicity at 20 µg/mL.

Compounds **1–7** were also tested for their activities against several plant pathogenic fungi, including *Corynespora cassicola*,²² *Collectotrichum gloeosporioides*,²³ and *Phytophthora colocasiae* (taro leaf blight)²⁴ using paper disk diffusion assays as described in the literature,^{25,26} but none was active at the concentration of 5 µg/disk for **1** and **2**, and 10 µg/disk for **3–7**, respectively.

The skeleton constructed as cyclooct-2-enone ring in compounds **1** and **2** is unusual in nature. The most closely related one is the only natural example of solalyratin B isolated from the whole plant of *Solanum lyratum*, recently.²⁷ Solalyratin B has the novel

cyclooct-2-enone ring as a side group whose NMR data correlated well with those of **1** and **2**.²⁷ Compounds **1** and **2** are the first unique natural products with the cyclooct-2-enone (cyclic eight-membered, -unsaturated ketone) skeleton. Compounds **1** and **2** could have the same precursor [(*E*)-4,8-dimethyl-nona-1,3,7-trien-2-ol] from farnesene as the known natural product (*E*)-4,8-dimethyl-nona-3,7-dien-2-one,²⁸ which underwent a nucleophilic cyclization followed by a series of dehydration and oxidation to afford compounds **1** and **2**. An oxocane derivative similar to (–)- heliannuol A²⁹ could also be generated through this pathway, but it was not detected in *Barleria lupulina*. Plausible biosynthetic pathways for compounds **1** and **2** are proposed as shown in Scheme 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This publication was made possible by grant number R01AT007022 (to DS and SC) from the National Center for Complementary and Alternative Medicine (NCCAM) at the National Institutes of Health. This research was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2A10005315). We also thank Baojun Yang and Chunshun Li for carrying out the anti-proliferative and anti-fungal assays.

References and notes

- Chopra, RN.; Nayar, SL.; Chopra, IC. Glossary of Indian Medicinal Plants (Suppl). Academic Publishers; New Delhi, India: 1968. p. 20
- Tiangburanatam, W. Dictionary of Thai Medicinal Plants. 4th. Prachoom Karn Pim; Bangkok, Thailand: 1996. p. 788-789.
- National Drug Committee. List of Herbal Medicinal Products AD. Chuoomnoom Sahakorn Karnkaset Publisher; Bangkok, Thailand: 2006. p. 59-61.
- Yoosook C, Panpisutchai Y, Chaichana S, Santisuk T, Reutrakul V. J Ethnopharmacol. 1999; 67:179–187. [PubMed: 10619382]
- Suba V, Murugesan T, Pal M, Mandal SC, Saha BP. Phytother Res. 2004; 18:925–929. [PubMed: 15597309]
- Wanikiat P, Panthong A, Sujayanon P, Yoosook C, Rossi AG, Reutrakul V. J Ethnopharmacol. 2008; 116:234–244. [PubMed: 18207341]
- Kanchanapoom T, Kasai R, Yamasaki K. Phytochemistry. 2001; 58:337–341. [PubMed: 11551561]
- Tuntiwachwuttikul P, Pancharoen O, Taylor WC. Phytochemistry. 1998; 49:163–166.
- Suksamrarn A. J Nat Prod. 1986; 49:179.
- Suksamrarn S, Wongkrajang K, Kirtikara K, Suksamrarn A. Planta Med. 2003; 69:877–879. [PubMed: 14598223]
- Widyowati R, Tezuka Y, Miyahara T, Awale S, Kadota S. Nat Prod Commun. 2010; 5:1711–1716. [PubMed: 21213964]
- Kim KH, Park YJ, Chung KH, Yip MLR, Clardy J, Senger D, Cao S. J Nat Prod. 2015; 78(2):320–324. [PubMed: 25611215]
- Chakyunglupulin A (I)*: amorphous powder; $[\alpha]_D^{25} + 25.1(c0.10, \text{MeOH})$; $UV_{\text{max}}(\text{MeOH}) \text{ nm (log): } 214 (3.10), 287 (1.26)$; **IR (KBr)**: $\nu_{\text{max}} = 3320, 2922, 2850, 1651, 1635, 1487, 1404, 1115, 1014 \text{ cm}^{-1}$; ^1H (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz): see Table 1; ESI-TOF-MS m/z 197 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; HRESI-TOF-MS m/z 197.1179 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ (calcd. for $\text{C}_{11}\text{H}_{17}\text{O}_3$, 197.1178).
- Kim KH, Choi SU, Kim YC, Lee KR. J Nat Prod. 2011; 74:54–59. [PubMed: 21182258]

15. To a stirred solution of **1** (0.5 mg) in CH₂Cl₂ (200 μ L) was added 4-(dimethylamino)pyridine (0.5 mg) and one drop of highly dried pyridine. Then, the mixture was treated with (*S*)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 2 μ L). The mixture was stirred at room temperature for 16 h. The reaction mixture was then passed through a silica gel Waters Sep-Pak Vac 6cc and eluted with *n*-hexane-EtOAc (10:1) to give the respective (*R*)-Mosher ester **1r**. Treatment of **1** (0.5 mg) with (*R*)-MTPA-Cl (2 μ L) as described above yielded the corresponding (*S*)-MTPA ester **1s**. Similarly, treatment of **2** with (*S*)- and (*R*)-MTPA-Cl afforded the respective Mosher esters **2r** and **2s**.
16. *Chakyunglupulin B* (**2**): amorphous powder; $[\alpha]_D^{25} - 22.0$ (c0.10, MeOH); UV_{max} (MeOH) nm (log): 214 (3.12), 287 (1.25); **IR** (**KBr**): $\nu_{\max}$ =3320, 2922, 2850, 1651, 1635, 1487, 1404, 1115, 1014 cm⁻¹; ¹H (CD₃OD, 600 MHz) and ¹³C NMR (CD₃OD, 150 MHz): see Table 1; ESI-TOF-MS *m/z* 197 [M – H₂O + H]⁺; HRESI-TOF-MS *m/z* 197.1176 [M – H₂O + H]⁺ (calcd. for C₁₁H₁₇O₃, 197.1178).
17. Houghton PJ. *Phytochemistry*. 1985; 24:819–826.
18. Takara K, Matsui D, Wada K, Ichiba T, Nakasone Y. *Biosci Biotechnol Biochem*. 2002; 66:29–35. [PubMed: 11866116]
19. Monais SKR, Teixeira AF, Torres ZE, Nunomura SM, Kanashiro EHY, Lindoso JAL, Yoshida M. *J Braz Chem Soc*. 2009; 20:1110–1118.
20. Xiong L, Zhu C, Li Y, Tian Y, Lin S, Yuan S, Hu J, Hou Q, Chen N, Yang Y, Shi J. *J Nat Prod*. 2011; 74:1188–1200. [PubMed: 21469695]
21. In SJ, Seo KH, Song NY, Lee DS, Kim YC, Baek NI. *Arch Pharm Res*. 2015; 38:26–34. [PubMed: 24676553]
22. Dixon LJ, Schlub RL, Pernezný K, Datnoff LE. *Phytopathology*. 2009; 99:1015–1027. [PubMed: 19671003]
23. Sangeetha CG, Rawal RD. *World J Agr Sci*. 2008; 4:717–720.
24. Aggarwal A, Kamlesh, Mehrotra RS. *Plant Disease Res*. 1993; 8:94–101.
25. Kumar SN, Nambisan B. *Appl Biochem Biotechnol*. 2014; 172:741–754. [PubMed: 24122628]
26. Joyce SA, Brachmann AO, Glazer I, Lango L, Schwar G, Clarke DJ, Bode HB. *Angew Chem Int Ed*. 2008; 47:1942–1945.
27. Zhang DW, Yang Y, Yao F, Yu QY, Dai SJ. *J Nat Med*. 2012; 66:362–366. [PubMed: 21898133]
28. Weyerstahl P, Christiansen C, Marschall H. *Flavour Frag J*. 1996; 11(1):15–23.
29. a Macias FA, Molinillo JMG, Varela RM, Torres A, Fronczek FR. *J Org Chem*. 1994; 59(26): 8261–8266. b Tuhina K, Bhowmik DR, Venkateswaran RV. *Chem Commun (Camb)*. 2002; (6): 634–635. [PubMed: 12120159]

Figure 1.
Structures of compounds **1** and **2**.

Figure 2.
Key COSY (bold line) and HMBC (arrows) correlations of compound **1**.

Figure 3.
 δ Values ($\delta_S - \delta_R$) in ppm of the two MTPA esters derived from **1** (a) and key NOE correlations in the NOESY spectrum of **1** (b)

Scheme 1.

Proposed biogenetic pathway for compounds **1** and **2**.

Table 1¹H (600 MHz) and ¹³C NMR (150 MHz) data of compounds **1** and **2** in CD₃OD.

No.	1		2	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}^a	δ_{H} (J in Hz)
1	183.6 s		185.4 s	
2	113.2 d	5.77, s	113.1 d	5.74, s
3	173.7 s		174.3 s	
4	88.3 s		88.8 s	
5 α	48.5 t	1.41, dd (12.0, 11.0)	46.1 t	1.73, overlap
5 β		2.46, ddd (11.0, 3.0, 2.5)		2.42, ddd (13.0, 2.5, 2.5)
6	64.8 d	4.09, m	67.0 d	4.20, m
7 α	50.2 t	1.28, overlap	47.7 t	1.51, dd (14.0, 3.0)
7 β		2.00, ddd (12.5, 2.5, 2.0)		1.97, ddd (14.0, 2.5, 2.0)
8	35.9 s		36.9 s	
9	25.0 q	1.28, s	26.7 q	1.45, s
10	30.0 q	1.30, s	30.8 q	1.26, s
11	25.5 q	1.58, s	27.2 q	1.75, s

^aThe assignments were based on ¹H-¹H COSY, HSQC, TOCSY, and HMBC experiments.