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A New Dioleate Compound from *Callistemon lanceolatus*

Ju Hee Kim, Jung Eun Kim, Hee Jung Bu, and Nam Ho Lee*

Department of Chemistry and Center for Cosmetic Sciences, Jeju National University, Jeju 690-756, Korea
**E-mail: namho@jejunu.ac.kr*
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Callistemon is a genus of 34 species of evergreen shrubs in the family Myrtaceae, most of which are endemic to Australia. These species are commonly named bottle brush trees because of their cylindrical brush-like flowers resembling a traditional bottle brush. In the southern parts of Korea including Jeju Island, *C. lanceolatus* is cultivated mainly as ornamental plants in gardens. A previous study disclosed that the essential oils from the leaves of *C. lanceolatus* possess antimicrobial and fungitoxic activities.[1] In addition, *anti*-inflammatory activities were observed in the methanol extract from these leaves.[2] Flavonoids isolated from the aerial parts displayed a preventive effect against Alzheimer's disease.[3] DPPH radical scavenging and elastase inhibition activities were also exhibited by the extracts of the stems.[4] In previous phytochemical studies, the extraction of *C. lanceolatus* leaves resulted in the isolation of triterpenes,[4,5] flavonoids,[3,6] phenolic compounds[7] and tannin derivatives.[8]

As a continuation of our efforts to find biologically active compounds from plants in Jeju Island,[9] further studies were conducted on the ethanol extract of *C. lanceolatus* stems. This led us to identify a new compound, 2-amino-2-ethylpropane-1,3-diyl dioleate (**1**), together with a known triterpene, ursolic acid 3-*O*-acetate (**2**).

Compound **1** showed a molecular ion peak at *m*/*z* 647.5846 (calcd *m*/z 647.5853) in the positive HR-FABMS spectrum, which is consistent with the molecular formula $C_{41}H_{77}O_4N$ (four unsaturations). A peak was observed at *m*/*z* 619.57 accounting for the fragmentation ion $[M+H-C_2H_5]^+$ in this FABMS spectrum. The IR spectrum exhibited a strong absorption peak at 1737.8 cm^{-1} indicating the presence of an ester functional group. The inspection of the ^{13}C and DEPT NMR spectra identified a total of 22 carbons, which include a carbonyl (δ 173.5), an olefin (δ 130.6, 130.7), a quaternary (δ 41.7) and two methyl (δ 8.1, 14.8) carbons. The rest of the signals corresponded to CH_2 carbons (Table 1).

In the ^1H NMR spectrum, the presence of the oleic acid moiety was inferred based on the distinctive peaks such as at δ 5.53 (2H, m, H-9', H-10'), 2.44 (2H, t, 7.5 Hz, H-2'), 2.14 (4H, m, H-8', H-11'), 1.72 (2H, dd, 7.5, 7.5 Hz, H-3') and 0.91 (3H, t, 7.5 Hz, H-18'). This oleate substructure was also supported by the HMQC-combined ^{13}C NMR data showing that the chemical shifts for C-1' to C-18' were all in accord with those in oleic acid methyl ester.[10] The *cis* configuration of the olefin at C-9' was further defined by the upfield resonances of the allylic carbons, C-8 (δ 28.0) and C-11' (δ 28.0). These allylic carbons appeared at δ 32.6 ppm for the ester of elaidic acid, the *trans*-isomer of oleic acid.[10] It is general in ^{13}C NMR that the allyilc methylenes in *cis*-olefins have higher resonance than those in *trans*-isomers due to shielding effects originated from their crowded environement.[11] The carbonyl peak (δ 173.7) of **1** is shifted up-field compared to that of the free oleic acid (δ ~180.9), which implies its presence as an ester linkage. Except for the signals corresponding to the oleate moiety, only four additional carbon peaks were observed in the ^{13}C NMR spectrum. In the ^1H NMR spectrum, one methylene (δ 1.65, s) and an isolated ethyl ($-CH_2CH_3$) group were identified besides the oleate structure. Interestingly, the inspection of the ^1H spectrum revealed that the integrated intensity of the protons in the oleate is two times greater than that of protons in the isolated ethyl group. For example, the integration the methyl protons (H_3-18') in the oleate appeared to be twice that of the ethyl (H_2-4 and H_3-5) protons. This strongly suggests that two equivalents of oleate are present symmetrically binding to the ethyl moiety. Finally, the connections of each subunit were established by means of a heteronuclear multiple bond correlation (HMBC) experiment (Figure 2). Two oleic acids are attached to the propane-1,3-diol through

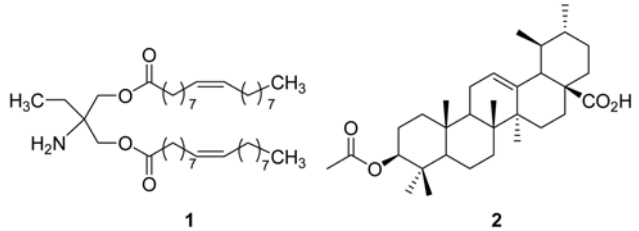

Figure 1. The structures of compounds **1** and **2** isolated from *C. lanceolatus*.

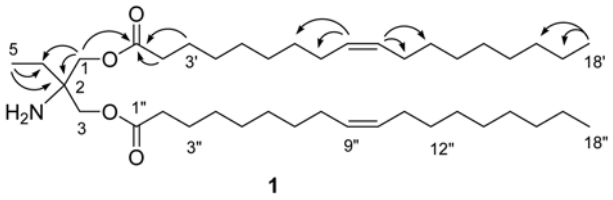

Figure 2. Key HMBC correlations in compound **1**.

ester bonds verified by the correlations of C-1' with H-1, H-2' and H-3'. The ethyl group is assigned to the C-2 position by the observation of HMBC cross peaks in H-4/C-1, H-4/C-2 and H-5/C-2. The presence of amino group at C-2 was determined based on the mass spectroscopic data. Therefore, compound **1** was identified as a new compound, 2-amino-2-ethylpropane-1,3-diyl dioleate.

From this ethanol extract of *C. lanceolatus*, compound **2** was isolated and characterized as a triterpene, ursolic acid 3-*O*-acetate. The elucidation of the structure of **2** was accomplished by obtaining its spectroscopic data and comparing it to the literature values.[12] Even though several triterpenoids have been isolated from *C. lanceolatus*, this is the first report of the isolation of **2** from this plant.

As the ethanol extract of *C. lanceolatus* exhibited *anti*-elastase activities, the corresponding activities were examined for the isolates **1** and **2**. Elastase is an enzyme causing the degradation of elastin, a fibrous protein maintaining elasticity in the skin dermis. Accordingly, elastase inhibitors have attracted attention as potential *anti*-wrinkle ingredients in cosmetic formulations.[13] The inhibitory activities were assayed on porcine pancreatic elastase (PPE) using N-Succ-$(Ala)_3$-*p*-nitroanilide (SANA) as the substrate by the spectrophotometric method. The release of *p*-nitroaniline was monitored by measuring the absorbance at 410 nm. The triterpene **2** showed moderate elastase inhibitory activity with an IC_{50} value of 17.3 μg/mL compared to that of oleanolic acid as a positive control (IC_{50} 3.0 μg/mL). On the other hand, compound **1** did not exhibit any such inhibitory activity in this test.

Experimental Section

Reagents and Instruments. The IR spectra were recorded on a Shimadzu Prestige-21 spectrophotometer. The UV spectra were obtained using a Biochrom Libra S22 spectrophotometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) the NMR spectra were recorded on a Bruker Ultrashield Plus 500 spectrometer. The chemical shift (δ) values are reported in ppm relative to the solvent used. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed using silica gel 60H (15 μm, Merck) and silica gel (0.063-0.2 mm), respectively. TLC was performed on silica gel GF_{254} plates (0.50 mm, Merck). Oleanolic acid, N-Succ-$(Ala)_3$-*p*-nitroanilide and porcine pancreatic elastase were purchased from Sigma.

Plant Material. The stems of *C. lanceolatus* were collected in Jeju Island in July, 2007. A voucher specimen (No. 263) is deposited at the Natural Product Chemistry Lab, Department of Chemistry, Jeju National University, Korea.

Extraction and Isolation. The shade-dried stems of *C. lanceolatus* (0.5 kg) were extracted two times with 70% ethanol with stirring at room temperature for 24 hrs. The combined filtrates were concentrated under reduced pressure to yield a gummy extract (22.0 g). The obtained extract was suspended in water and partitioned into *n*-hexane (*n*-Hex), ethyl acetate (EtOAc), *n*-butane and water soluble fractions successively. A portion of the EtOAc-soluble fraction (2.0 g) was subjected to vacuum liquid chromatography on silica gel using step-gradients (*n*-Hex/EtOAc and EtOAc/MeOH) to give eight fractions (fr. I-VIII). The fourth fraction (fr. IV, 640 mg), eluted with 30% *n*-Hex in EtOAc, was subjected to silica gel column chromatography with a $CHCl_3$/MeOH gradient solution to afford six fractions (fr. IV-1 to IV-6). The most nonpolar fraction (IV-1, 52 mg), eluted with 100% $CHCl_3$, was identified as compound **1**. Compound **2** (16.2 mg) was obtained from fraction III by using silica gel column chromatography with a solvent gradient (*n*-Hex/EtOAc).

Elastase Inhibition Activity Assay. The procine pancreatic elastase (PPE) inhibition activities were determined with N-Succ-$(Ala)_3$-*p*-nitroanilide (SANA) as the substrate according to the literature method with some modifications.[14] Briefly, PPE (0.1 mg/mL, 0.01 mL) and 12.5 mM SANA (0.005 mL) were added to Tris-HCl buffer (0.2 M, 0.165 mL) containing the test sample (0.2 mL). The test mixture (0.2 mL) was incubated for 15 min at 25 ºC and the absorption due to the formation of *p*-nitroaniline was monitored at 405 nm. The same mixture, except for the plant extract, was used as a control. Oleanolic acid was used as a positive control. Each treatment was replicated three times. The percent inhibition of elastase activity was calculated as follows:

$$\% \text{ inhibition} = [1 - (Abs_{sample} - Abs_{blank}) / (Abs_{control} - Abs_{c\text{-}blank})] \times 100$$

Table 1. 1D and 2D NMR data for **1** in pyridine-d_5

No	δ_C (mult)[a]	δ_H (int, mult, *J* in Hz)	HMBC (H → C)
1 & 3	64.5 (t)	4.37 (4H, s)	C-2, C-4, C-1', C-1"
2	41.7 (s)	-	
4	24.0 (t)	1.65 (2H, q, 7.5)	C-1, C-4
5	8.1 (q)	0.99 (3H, t, 7.5)	C-2, C-4
1' & 1"	173.7 (s)	-	
2' & 2"	34.7 (t)	2.44 (4H, t, 7.5)	C-1', C-1"
3' & 3"	25.7 (t)	1.72 (4H, dd, 7.5)	C-1', C-1"
4' & 4"	30.3 (t)[d]	1.29-1.37 (4H, m)	
5' & 5"	29.9 (t)[d]	1.29-1.37 (4H, m)	
6' & 6"	30.4 (t)[b]	1.29-1.37 (4H, m)	
7' & 7"	30.0 (t)	1.38-1.45 (4H, m)	
8' & 8"	28.0 (t)	2.14 (4H, m)	
9' & 9"	130.6 (d)[c]	5.53 (2H, m)	C-7', C-8', C-7", C-8"
10' & 10"	130.7 (d)[c]	5.53 (2H, m)	C-11', C-12', C-11", C-12"
11' & 11"	28.0 (t)	2.14 (4H, m)	
12' & 12"	30.0 (t)	1.38-1.45 (4H, m)	
13' & 13"	30.6 (t)[b]	1.29-1.37 (4H, m)	
14' & 14"	29.8 (t)[d]	1.29-1.37 (4H, m)	
15' & 15"	29.8 (t)[d]	1.29-1.37 (4H, m)	
16' & 16"	32.6 (t)	1.29-1.37 (4H, m)	
17' & 17"	23.4 (t)	1.29-1.37 (4H, m)	
18' & 18"	14.8 (q)	0.91 (6H, t, 7.5)	C-16', C-17', C-16", C-18"

[a]Determined by DEPT experiments. [b,c,d]Values are respectively interchangeable.

where Abs_{sample} is the absorbance of the experimental sample, Abs_{blank} is the absorbance of the test sample in the buffer, $Abs_{control}$ is the absorbance of SANA and elastase in the buffer, and $Abs_{c\text{-}blank}$ is the absorbance of SANA in the buffer. IC_{50} indicates the concentration of the sample when the enzyme activity was inhibited by 50%, as determined by linear curve fitting.

2-Amino-2-ethylpropane-1,3-diyl Dioleate (1). Viscous liquid; UV (CH_3OH): λ_{max} 291 nm; IR (cm^{-1}): 1737.8, 2950.0; ^1H and ^{13}C NMR data: Table 1; HR-FABMS (positive): *m*/*z* 647.5846 $[M]^+$ (calcd for $C_{41}H_{77}O_4N$ 647.5853, Δ −0.7 mmu).

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References

1. Sudhakar, M.; Raju, D. B. *Int. J. Chem. Sci*. **2005**, *3*, 513.
2. Kumar, S.; Kumar, V.; Prakash, O. M. *Asian Pac. J. Trop. Biomed*. **2011**, *1*, 177.
3. Park, S. Y.; Lim, J. Y.; Jeong, W.; Hong, S. S.; Yang, Y. T.; Hwang, B. Y.; Lee, D. *Planta Med*. **2010**, *76*, 863.
4. Kim, J. H.; Byun, J. C.; Bandi, A. K. R.; Hyun, C-G.; Lee, N. H. *J. Med. Plant. Res*. **2009**, *3*, 914.
5. (a) Younes, M. E. G. *Australian J. Chem*. **1975**, *28*, 221. (b) Jeong, W. S.; Hong, S. S.; Kim, N. H.; Yang, Y. T.; Shin, Y. S.; Lee, C.; Hwang, B. Y.; Lee, D. H. *Arch. Pharm. Res*. **2009**, *32*, 845.
6. Mahmoud, II.; Moharram, F. A.; Marzouk, M. S. A.; Linscheid, M. W.; Saleh, M. I. *Pharmazie* **2002**, *57*, 494.
7. Lounasmaa, M.; Puri, H-S.; Widen, C-J. *Phytochemistry* **1977**, *16*, 1851.
8. Marzouk, M. S. A. *Phytochem. Anal*. **2008**, *19*, 541.
9. (a) Kim, S. S.; Kim, J. E.; Hyun, C.-G.; Lee, N. H. *Nat. Prod. Commun.* **2011**, *6*, 1193. (b) Oh, T. H.; Baik, J. S.; Yoo, E.-S.; Kang, H. K.; Lee, N. H. *Bull. Korean Chem. Soc*. **2011**, *32*, 3175.
10. Vieville, C.; Mouloungui, Z.; Gaset, A. *Chem. Phys. Lipids* **1995**, *75*, 101.
11. Crews, P.; Rodriguez, J.; Jaspars, M. *Organic Structure Analysis*, Oxford: New York, 1998; p 78.
12. Fujita, R.; Duan, H.; Takaishi, Y. *Phytochemistry* **2000**, *53*, 715.
13. Kim, Y. H.; Kim, K. H.; Han, C. S.; Yang, H. C.; Park, S. H.; Jang, H. J.; Kim, J. W.; Choi, Y. S.; Lee, N. H. *J. Cosmet. Sci*. **2010**, *61*, 211.
14. Kraunsoe, J. A. E.; Claridge, T. D. W.; Lowe, G. *Biochemistry* **1996**, *35*, 9090.