Bioorganic & Medicinal Chemistry Letters 26 (2016) 4950–4954

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Sesquiterpenes from the roots of *Lindera strychnifolia* with inhibitory effects on nitric oxide production in RAW 264.7 cells

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ARTICLE INFO

Article history:
Received 29 April 2016
Revised 18 August 2016
Accepted 5 September 2016
Available online 7 September 2016

Keywords:
Lindera strychnifolia
Lauraceae
Sesquiterpene
Linderolides N–T
Anti-inflammation

ABSTRACT

Seven new sesquiterpenes, linderolides N–T (**1**–**7**), along with nine known compounds, were isolated from roots of *Lindera strychnifolia* (Lauraceae). Their structures were established by extensive spectroscopic analysis. The relative and absolute configurations were determined by NOESY and CD analysis, respectively. Among the isolated compounds, two new compounds, linderolide O (**2**) and linderolide P (**3**) inhibited lipopolysaccharide-stimulated nitric oxide production in murine RAW 264.7 macrophage cells, with IC_{50} values of 6.3 and 9.6 μM, respectively.

Inflammation, one of the most common diseases, is a complex self-defense mechanism triggered by harmful stimuli such as pathogens and damaged tissues.[1] Macrophages play a central role in inflammatory responses by the release of numerous cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-12, and cytotoxic and inflammatory molecules such as nitric oxide (NO). Although inflammation is part of defense response, excessive and chronic inflammation might lead to the development of some chronic inflammation diseases, like atherosclerosis, rheumatoid arthritis, cancer, and allergies.[2] Therefore, inhibition of overstimulated inflammatory cytokines and NO is potential therapeutic target for inflammatory disease.[3]

Lindera strychnifolia Villar (Lauraceae) is an evergreen shrub which is widely distributed in Asia. Lindera Radix has strong fragrances and traditionally has been used to promote the flow of Qi to relive pain and for the treatment of kidney deficiencies such as pollakisuria.[4] Recent study also reported antioxidant,[5] anti-diabetic[6] and anti-inflammatory[7] effect of Lindera Radix. Sesquiterpenes are major constituents of this plant and alkaloids and tannin are also isolated.[8]

We previously isolated nineteen sesquiterpenes from the roots of *Lindera strychifolia* and reported the cytotoxicity against HSC-T6 hepatic stellate cells.[9] In a continuation of our research, *L. strychnifolia* inhibited lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in RAW264.7 macrophage cells. Further extensive chromatographic separation of *L. strychnifolia* afforded additional sixteen compounds (**1**–**16**) (Fig. 1).[10]

Compound **1**[11] was isolated as a white powder. The ESIMS spectrum showed a pseudomolecular ion peak at *m*/*z* 249 $[M+H]^+$, and the molecular formula was established as $C_{15}H_{20}O_3$ by HRESIMS (*m*/*z* 249.1485 $[M+H]^+$, calcd 249.1875) with 6 degrees of unsaturation. The IR spectrum exhibited absorptions at 3440 and 1741 cm^{-1}, which were assignable to hydroxyl group and γ-lactone, respectively. The ^1H NMR spectrum showed the signals for three methyl groups at δ_H 1.22 (s), 1.29 (s) and 1.82 (d, J = 2.0 Hz) and one hydroxymethine at δ_H 4.77 (1H, dd, J = 12.0, 6.0 Hz) (Table 1). The ^{13}C NMR together with HSQC and HMBC spectrum showed 15 carbon signals for one ester carbonyl carbon at δ_C 175.2, one tetrasubstituted double bond at δ_C 119.2 and 163.2, three methyl groups at δ_C 8.3, 23.7 and 25.4, three methylenes at δ_C 7.0, 20.4 and 43.7, four methines at δ_C 28.0, 29.4, 49.5 and 78.0, and two quaternary carbons at δ_C 42.2 and 81.3 (Table 2). The characteristic shielded hydrogen signals at δ_H 0.19 and δ_H 0.53 in the ^1H NMR spectrum together with its shielded corresponding carbon signal at δ_C 7.0 in the HSQC spectrum suggests **1** as an 1,3 cycloeudesm-7(11)-en-12,8-olide.[12] In the HMBC spectrum, correlations were observed between δ_H 1.29 (Me-15) and δ_C 81.3, which suggested the addition of a hydroxyl group to C-4.[9] Taken together, compound **1** was suggested as 4-hydroxy-1,3-cycloeudesma-7 (11)-en-12,8-olide. Compound **2**[12] was purified as a white amorphous powder, and gave HREIMS at *m*/*z* 263.1637 $[M+H]^+$, consistent with a molecular formula of $C_{16}H_{22}O_3$ (calcd 263.1641 $[M+H]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **2** with those

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http://dx.doi.org/10.1016/j.bmcl.2016.09.012
0960-894X/

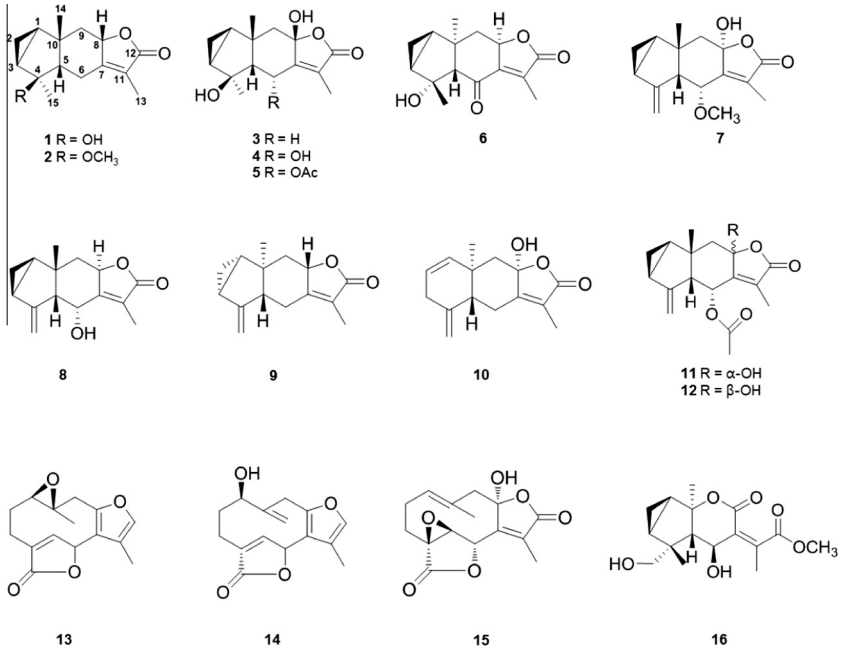

Figure 1. Chemical structures of compounds **1**–**16** from the roots of *L. strychnifolia.*

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**7** in $CDCl_3$

H	1	2	3	4	5	6	7
1	1.35 m	1.29 m	1.31 m	1.35 m	1.34 m	1.27 m	1.42 m
2α	0.19 m[a]	0.50 m	0.50 m	0.53 m	0.51 m	0.18 m	0.78 m[b]
2β	0.53 m[a]	0.59 m	0.59 m	0.69 m	0.62 m	0.61 m	0.85 m[b]
3	1.34 m	1.31 m	1.42 m	1.39 m	1.41 m	1.45 m	2.05 m
5	1.33 m	1.71 (d 9.0)	1.67 (d 8.0)	2.02 (d 8.0)	2.16 (d 7.7)	1.97 br s	3.51 (d 10.4)
6α	2.77 (d 16.0)	2.79 (d 16.0)	2.76 (d 15.0)	5.14 (dd 1.6, 4.8)	6.03 (dd 7.7, 2.1)		4.09 (d 10.4)
6β	2.34 m	2.37 (ddt 16.0, 8.5, 1.5)	2.54 (ddd 15.0, 8.0, 1.5)				
8	4.77 (dd 12.0, 6.0)	4.75 (dd 6.0, 12.5)				5.15 m	
9α	1.77 (d 12.0)	1.52 (t 12.5)	1.90 (d 14.0)	1.96 (d 14.4)	1.92 (d 14.0)	2.24 (t 12.4)	2.29 (d 14.0)
9β	2.31 m	2.28 m	2.27 (d 14.0)	2.24 (dd 14.4, 1.2)	2.30 (d 14.0)	2.47 (ddd 12.4, 6.0, 1.6)	2.55 (d 14.0)
13	1.82 (d 2.0)	1.83 (t 2.0)	1.87 (d 1.5)	2.10 (d 2.0)	1.98 (d 1.4)	2.06 (d 2.0)	2.04 s
14	1.22 s	1.21 s	1.35 s	1.33 s	1.37 s	1.25 s	0.55 s
15	1.29 s	1.12 s	1.18 s	1.45 s	1.40 s	1.47 s	5.02 br s, 5.11 br s
OCH_3		3.30 s					3.45 s
$COCH_3$					2.20 s		

[a,b] Exchangeable.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**7** in $CDCl_3$

C	1	2	3	4	5	6	7
1	28.0 d	29.4 d	28.2 d	29.2 d	29.5 d	29.3 d	29.3 d
2	7.0 t	5.4 t	5.3 t	5.4 t	5.3 t	6.8 t	6.8 t
3	29.4 d	22.8 d	29.7 d	28.2 d	28.8 d	29.7 d	23.7 d
4	81.3 s	84.9 s	80.7 d	81.5 d	80.7 d	84.8 s	149.6 s
5	49.5 d	50.3 d	51.4 d	56.4 d	54.4 d	64.2 d	61.1 d
6	20.4 t	21.2 t	19.7 t	67.9 d	69.2 d	197.4 s	71.5 d
7	163.2 s	162.2 s	159.4 s	158.7 s	155.2 s	153.8 s	155.0 s
8	78.0 d	78.0 d	103.0 s	103.7 s	103.6 s	78.7 d	105.4 s
9	43.7 t	42.9 t	45.9 t	45.2 t	45.5 t	41.2 t	50.5 t
10	42.2 s	40.8 s	40.7 s	40.7 s	41.6 s	42.5 s	37.3 s
11	119.2 s	120.2 s	122.9 s	123.3 s	123.0 s	130.6 s	129.8 s
12	175.2 s	174.8 s	171.8 s	171.6 s	171.1 s	173.3 s	171.1 s
13	8.3 q	8.5 q	8.8 q	9.1 q	9.0 q	9.7 q	9.6 q
14	23.7 q	24.3 q	25.3 q	25.2 q	25.3 q	24.6 q	21.4 q
15	25.4 q	20.7 q	26.0 q	27.0 q	26.6 q	26.2 q	107.2 q
OCH_3		51.0 q					57.3 q
$COCH_3$					170.0 s		
$COCH_3$					21.0 q		