*SHORT REPORT*

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# Callignan A, A New Neolignan from the Leaves of *Callicarpa kwangtungensis*

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**Abstract:** In the current study, a new lignan (**1**), named callignan, as well as two known ones (**2** and **3**), were obtained from the EtOAc extract of the leaves of *Callicarpa kwangtungensis*. The structure elucidation of **1** was conducted by detailed interpretation of the 1D and 2D NMR spectroscopic data. Optical rotation of compound **1** and ECD data revealed that the compound was a racemic compound. Compounds **1**–**3** showed moderate effects with $IC_{50}$ values of 46, 29, and 42 μM on NO production in LPS-induced RAW 264.7 cell inflammation model.

**Keywords:** *Callicarpa kwangtungensis*; callignan A; neolignan; 

## 1. Plant Source

The leaves of *Callicarpa kwangtungensis* were collected from Conghua, Guangdong province, in June of 2021. The species was identified by Prof. Ying Lu at First People's Hospital of Linping District, Hangzhou of China. A voucher specimen of this species was deposited in the Herbarium of Sun Yat-sen University (Herbarium number:SYS00235653)

## 2. Previous Studies

*C. kwangtungensis* Chun. is a representative medicinal plant of the genus *Callicarpa*, which is mainly distributed in Guangdong, Jiangxi, Fujian, and Hubei Provinces. It has been included in the Chinese Pharmacopoeia. The stems and leaves have obvious effects such as astringency and hemostasis, scattered and stasis detumescence, heat clearing and detoxification. The literature reveal that the chemical constituent of this plant consist of phenylethanoid glycosides [1-5], triterpenoids [6, 7], norneolignans [8], and diterpenoids [9]. Some compounds were reported to show obvious activity, particularly, forsythiaside B, major ingredient of *C. kwangtungensis*, can inhibit myocardial fibrosis via down regulating TGF-β1/Smad signaling pathway [10].

## 3. Present Study

The leaves of *C. kwangtungensis* (300 g) were ground and were extracted for three times using the solvent ethanol to give a crude extract. The crude extract (35 g) was partitioned with EtOAc/$H_2O$ to afford the EtOAc extract (10.3 g), which was further separated by repeated column chromatography (CC) including silica gel, ODS silica gel, and RP-HPLC to give a new lignan (**1**) and two known ones

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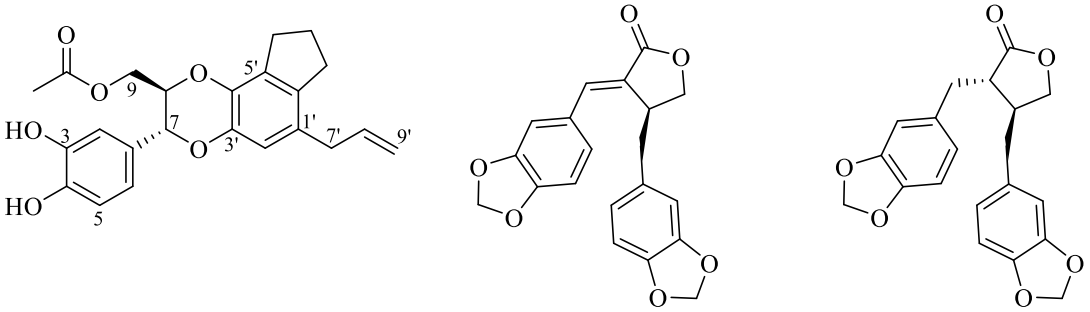
(**2** and **3**). Herein, the separation, structure identification, and the biological evaluation of these compounds were presented.

Callignan A (**1**): Colorless oil; $[\alpha]^{25}_{D}$ 0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 204 (4.98), 243 (2.79) nm; $^{1}$H and $^{13}$C NMR data, see Table 1; HRESIMS *m*/*z* 357.1334 $[M + H]^+$ (calcd. for $C_{20}H_{21}O_6^+$, 357.1333), *m*/*z* 379.1157 $[M + Na]^+$ (calcd. for $C_{20}H_{20}O_6Na^+$, 379.1152).



**1** **2** **3**

**Figure 1**. Structures of compounds **1**–**3** from *C. kwangtungensis*

**Table 1.** $^{1}$H and $^{13}$C NMR Data of **1** in Methanol-$d_4$[a]

| No. | **1** $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1 | | 129.1, C |
| 2 | 6.83, d (1.8) | 115.5, CH |
| 3 | | 147.4, C |
| 4 | | 146.8, C |
| 5 | 6.81, d (7.8) | 116.5, CH |
| 6 | 6.74, dd (7.8, 1.8) | 120.4, CH |
| 7 | 4.76, d (7.7) | 77.9, CH |
| 8 | 4.18, ddd (7.7, 4.3, 2.8) | 77.1, CH |
| 9 | a 4.23, dd (11.8, 2.8)<br>b 3.92, dd (11.8, 4.3) | 64.4, $CH_2$ |
| 1′ | | 134.5, C |
| 2′ | 6.73, d (1.7) | 120.4, CH |
| 3′ | | 145.1, C |
| 4′ | | 142.8, C |
| 5′ | 6.82, d (8.3) | 117.8, CH |
| 6′ | 6.68, dd (8.3, 1.7) | 122.7, CH |
| 7′ | 3.27, d (6.7) | 40.5, CH |
| 8′ | 5.93, ddd (15.8, 8.6, 7.0) | 139.1, CH |
| 9′ | a 5.00, d (8.6)<br>b 5.04, d (15.8) | 115.7, $CH_2$ |
| | 2.03, s | 20.5, $CO\underline{CH_3}$ |
| | | 172.4, CO |

[a] $^{1}$H NMR (400 MHz); $^{13}$C NMR (100 MHz)

Compound **1** was obtained as a colorless oil, its chemical formula was assigned to be $C_{20}H_{20}O_6$ by the HRESIMS (*m*/*z* 357.1334 $[M + H]^+$, calcd. for $C_{20}H_{21}O_6^+$, 357.1333) and $^{13}$C NMR spectra, suggesting the presence of 11 index of hydrogen deficiency. Summation of the integrals in the $^{1}$H NMR spectrum of **1** in methanol-$d_4$ revealed the presence of two hydroxyl groups. Further analyses of the $^{1}$H NMR spectrum of **1** suggested the presence of six protons for two 1,2,4-trisubstituted benzene

moieties A [$\delta_H$ 6.83 (1H, d, *J* = 1.8 Hz), 6.81 (1H, d, *J* = 7.8 Hz); 6.74 (1H, dd, *J* = 7.8, 1.8 Hz)] and B [$\delta_H$ 6.73 (1H, d, *J* = 1.7 Hz), 6.82 (1H, d, *J* = 8.3 Hz); 6.68 (1H, dd, *J* = 8.3, 1.7 Hz)], four oxygen-bearing protons [$\delta_H$ 4.76 (1H, d, *J* = 7.7 Hz); 4.18 (1H, ddd, *J* = 7.7, 4.3, 2.8 Hz); 4.23 (1H, dd, *J* = 11.8, 2.8 Hz); 3.92 (1H, dd, *J* = 11.8, 4.3 Hz)] including two geminally coupled protons ($\delta_H$ 4.23, 3.92) for a methyene, three protons for a monosubstituted terminal double bond [$\delta_H$ 5.93 (ddd, *J* = 15.8, 8.6, 7.0 Hz, 1H), 5.00 (d, *J* = 8.6 Hz, 1H), 5.04 (d, *J* = 15.8 Hz, 1H)], a doublet integrated for two protons for a methylene [$\delta_H$ 3.27 (d, *J* = 6.7 Hz)], and a methyl [$\delta_H$ 2.03 (3H, s)] of an acetyl group.

The $^{13}$C NMR data of **1** suggested the presence of 20 carbons, which could be attributed with the aid of HSQC spectrum to twelve aromatic carbons for two benzene rings, two olefinic carbons for a double bond ($\delta_C$ 139.1, 115.7), two sp$^3$ methylenes including one oxygenated ($\delta_C$ 64.4), two oxygen-bearing methines ($\delta_C$ 77.9, 77.1), and an acetyl group ($\delta_C$ 20.5, 172.4).

The terminal double bond was connected to the methylene ($\delta_H$ 3.27, 40.5) via the COSY relationship to form an allyl group, which was linked to the benzene moiety B at C-1′ ($\delta_C$ 134.5) by the HMBC correlations from $H_2$-7′ ($\delta_H$ 3.27) to C-1′, C-2′ ($\delta_C$ 120.4), C-6′ ($\delta_C$ 122.7).

The oxygenated protons H-7 ($\delta_H$ 4.76), H-8 ($\delta_H$ 4.18), and H-9 ($\delta_H$ 3.92, 4.23) were in a spin system as evidenced by the cross-peaks in the COSY spectrum. An acetoxy group was further located at C-9 by the HMBC correlations from $H_2$-9 to the carbonyl carbon ($\delta_C$ 172.4) of the acetyl group, this fragment was attached to the benzene moiety A at C-1 ($\delta_C$ 129.1) based on the cross-peaks (H-7/C-1) in the HMBC spectrum.

The above analyses led to the construction of the two phenylpropanoid fragments (**a** and **b**) as in figure 2. The chemical shifts of C-3 and C-4 in fragment **a** were almost identical to those of the known lignan jatrophasin C [11], revealing that the oxygen-containing substituents at C-3 and C-4 were both hydroxyl groups. As the functional groups accounted for 10 indices of hydrogen deficiency, requiring the presence of an additional ring. HMBC correlations of H-7 to C-3′ ($\delta_C$ 145.1) and the remaining ring suggested that the two fragments were connected via a dioxane moiety as shown in Figure 2.

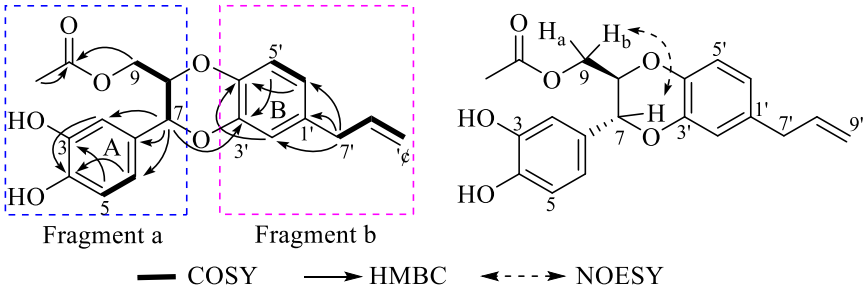



**Figure 2.** Key HMBC, COSY, and NOESY correlations of compound **1**

The relative configuration of the protons H-7 and H-8 were assigned to be *trans* according to the coupling constant $J_{7,8}$ = 7.7 Hz [11], which was supported by the NOESY correlations of H-9b ($\delta_H$ 3.92)/H-7 ($\delta_H$ 4.76). The optical rotation and ECD data of compound **1** were negligible, which suggested the racemic nature of **1**. Thus, the structure of **1** was established as depicted and it was named (±)-callignan A.

Compounds **2** and **3** were identified to be savinin (**2**) [12], hinokinin (**3**) [13] by comparing their NMR data and optical rotations with those in the literature.

The phenylpropanoids and lignans showed various activities including anti-inflammatory activity [14-18], the inhibitory effects on NO production in LPS-induced RAW 264.7 cell inflammation model were assayed following the procedures in literature [16]. As a result, compounds **1**–**3** showed moderate effects with $IC_{50}$ values of 46, 29, and 42 μM respectively, which are comparable to the positive control indomethacin (38 μM)

## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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## References

[1] E. L. Xie, G. P. Zhou, T. F. Ji, J. Wu and G. P. Yuan (2009). A novel phenylpropanoid glycoside from *Callicarpa kwangtungensis* Chun, *Chin. Chem. Lett.* **20**, 827-829.

[2] J.-N. Zheng, J.-Y. Zhuo, J. Nie, Y.-L. Liu, B.-Y. Chen, A.-Z. Wu and Y.-C. Li (2021). Phenylethanoid glycosides from *Callicarpa kwangtungensis* chun attenuate TNF-α-induced cell damage by inhibiting NF-κB pathway and enhancing Nrf2 pathway in A549 cells, *Front. Pharmacol.* **12**, 693983.

[3] J. Sun, L. Chen, P. Jiang, B. Duan, R. Wang, J. Xu, W. Liu, Y. Xu, Z. Xie, F. Feng and W. Qu (2020). Phenylethanoid glycosides of *Callicarpa kwangtungensis* Chun exert cardioprotective effect by weakening Na+-K+-ATPase/Src/ERK1/2 pathway and inhibiting apoptosis mediated by oxidative stress and inflammation, *J. Ethnopharmacol.* **258**, 112881.

[4] A. Wu, Z. Yang, Y. Huang, H. Yuan, C. Lin, T. Wang, Z. Zhao, Y. Zhou and C. Zhu (2020). Natural phenylethanoid glycosides isolated from *Callicarpa kwangtungensis* suppressed lipopolysaccharide-mediated inflammatory response via activating Keap1/Nrf2/HO-1 pathway in RAW 264.7 macrophages cell, *J. Ethnopharmacol.* **258**, 112857.

[5] Y. Xu, X. Jiang, J. Xu, W. Qu, Z. Xie, R.-W. Jiang and F. Feng (2021). A previously undescribed phenylethanoid glycoside from *Callicarpa kwangtungensis* Chun acts as an agonist of the Na/K-ATPase signal transduction pathway, *Phytochemistry* **181**, 112577.

[6] M.-M. Yuan, R.-J. Zhong, G. Chen, G.-P. Zhou, H.-Z. Fu and Q.-W. Yan (2015). Two new triterpenoids from *Callicarpa kwangtungensis*, *J. Asian Nat. Prod. Res.*, **17**, 138-142.

[7] G.-P. Zhou, Y. Yu, M.-M. Yuan, T. Ji, H.-Z. Fu and R.-J. Zhong (2015). Four new triterpenoids from *Callicarpa kwangtungensis*, *Molecules* **20**, 9071-9083.

[8] H.-Y. Bi, C.-L. Xu, H.-Z. Fu, Z.-Q. Zhou, R.-J. Zhong, M. Dai and D. Wang (2020). Two new norneolignans from *Callicarpa kwangtungensis*, *Nat. Prod. Res.* **34**, 197-203.

[9] J. Xu, S. Li, X. Sun, J. Ma, F. Liu, L. Tong, D. Lee, Y. Ohizumi, M. Tuerhong and Y. Guo (2016). Diterpenoids from *Callicarpa kwangtungensis* and their NO inhibitory effects, *Fitoterapia* **113**, 151-157.

[10] J. Sun, J. Zhu, L. Chen, B. Duan, R. Wang, M. Zhang, J. Xu, W. Liu, Y. Xu, F. Feng and W. Qu (2021). Forsythiaside B inhibits myocardial fibrosis via down regulating TGF-β1/Smad signaling pathway, *Eur. J. Pharmacol.* **908**, 174354.

[11] X. Li, L. Li, J. Wang, T. Wang and L. Wang (2014). Two new lignans with antioxidative activities from *Jatropha curcas*, *Nat. Prod. Res.* **28**, 1985-1991.

[12] S. Lee, H. H. Yoo, X. L. Piao, J. S. Kim, S. S. Kang and K. H. Shin (2005). Anti-estrogenic activity of lignans from *Acanthopanax chiisanensis* root, *Arch. Pharmacal Res.* **28**, 186-189.

[13] Y. Xia, J. You, Y. Zhang and Z. Su (2009). Synthesis, anti-virus and anti-tumor activities of dibenzylbutyrolactone lignans and their analogues, *J. Chem. Res.* 565-569.

[14] Y. Z. Wang, W. Zhang, L. Ren, J. Sun and D. B. Zhang (2021). Trimacoside A, a high molecular weight antioxidant phenylpropanoid glycoside from *Tricyrtis maculata*, *Rec. Nat. Prod.* **15**, 194-201.

[15] V. Popovic, A. Heyerick, S. Petrovic, S. Van Calenbergh, I. Karalic, M. Niketic and D. Deforce (2013). Cytotoxic activity of *Laserpitium latifolium* L. extract and its daucane and phenylpropanoid constituents, *Rec. Nat. Prod.* **7**, 245-249.

[16] Y. Liu, X. Yin, Y. P. Sun, Y. Liu, D. Lu, Y. Y. Zhou, M. A. Adnan, J. Pan, W. Guan, B. Y. Yang and H. X. Kuang (2021). A new phenylpropanoid from the roots of *Solanum melongena* L. and evaluation of anti-inflammatory activity, *Rec. Nat. Prod.* **15**, 261-266.

[17] Y. Chi, H. Zhou, H.-W. He, Y.-D. Ma, B. Li, D. Xu, J.-M. Gao and G. Xu (2021). Total synthesis and anti-tobacco mosaic virus activity of the furofuran lignan (±)-phrymarolin II and its analogues, *J. Nat. Prod.* **84**, 2937-2944.

[18] X. Yang, Y. Guo, T. J. Tse, S. K. Purdy, R. Mustafa, J. Shen, J. Alcorn and M. J. T. Reaney (2021). Oral pharmacokinetics of enriched secoisolariciresinol diglucoside and its polymer in rats, *J. Nat. Prod.* **84**, 1816-1822.