Organic LETTERS

Letter

pubs.acs.org/OrgLett

Icacinlactone H and Icacintrichantholide from the Tuber of *Icacina trichantha*

Ming Zhao,*[,†] Monday M. Onakpa,[†,‡] Bernard D. Santarsiero,[§] Xiao-Jun Huang,[∥] Xiao-Qi Zhang,[†,∥] Jia Chen,[⊥] Jian-Jun Cheng,[†] Richard Longnecker,[⊥] and Chun-Tao Che[†]

[†]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States

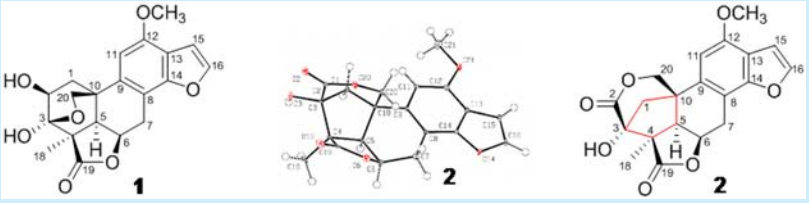
[‡]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja, Abuja 920001, Nigeria

[§]Center for Pharmaceutical Biotechnology, and Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, Illinois 60607, United States

[∥]Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China

[⊥]Department of Microbiology and Immunology, The Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, United States

Supporting Information



ABSTRACT: The 17-norpimarane diterpene is a small group of natural products. A new member, icacinlactone H (**1**), and a new rearranged 17-norpimarane with an unprecedented carbon skeleton, icacintrichantholide (**2**), were isolated from *Icacina trichantha*. Their structures including the absolute configuration were elucidated by spectroscopic analysis, single-crystal X-ray diffraction, and electronic circular dichroism analysis. A plausible biogenetic pathway for **1** and **2** is proposed. Both **1** and **2** showed no significant activity against herpes simplex viruses HSV-1 and HSV-2 as well as the Epstein−Barr virus at 50 μM.

Icacina trichantha Oliv. (Icacinaceae) is a traditional herbal medicine used in Nigeria and other regions of western Africa. The tuber of this plant is often prescribed by herbalists for the treatment of food poisoning, constipation, and malaria and is also a common household first-aid medicine for emergency treatment of food poisoning.[1,2] Recent reports on *I. trichantha* showed its antihyperglycemic, anticonvulsion, sedative, analgesic, and antimicrobial properties.[3−5] Previous phytochemical studies have led to the discovery of a few 17-norpimaranes, (9βH)-17-norpimaranes, and (9βH)-pimaranes from the genus of *Icacina* (*I. claessensis*, *I. mannii*, *I. guesfeldtii*, and *I. trichantha*).[6−10] During our continuing search for bioactive natural products, a new 17-norpimarane, icacinlactone H (**1**), and a new rearranged 17-norpimarane with an unprecedented carbon skeleton, icacintrichantholide (**2**), were isolated from *I. trichantha* (Figure 1). We herein report the isolation and structural elucidation of **1** and **2**. A plausible biogenetic pathway is also proposed. Compounds **1** and **2** were evaluated for their activity against herpes simplex viruses HSV-1 and HSV-2 as well as the Epstein−Barr virus.

The EtOAc-soluble fraction of an 80% MeOH extract of the tuber of *I. trichantha* was subjected to silica gel column chromatography followed by semipreparative HPLC separation on a C_{18} column to afford compounds **1** and **2**.

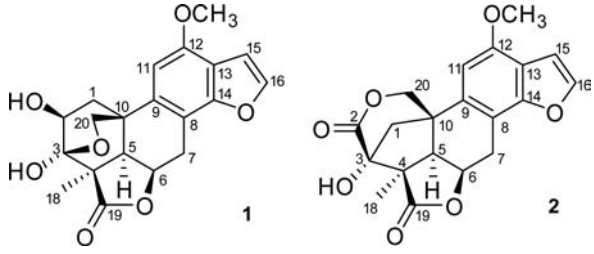


Figure 1. Structures of compounds **1** and **2**.

Icacinlactone H (**1**) was obtained as a colorless amorphous powder. A molecular formula of $C_{20}H_{20}O_7$ with 11 indices of hydrogen deficiency was suggested by HRESIMS (m/z 373.1256 $[M + H]^+$, calcd for $C_{20}H_{21}O_7$, 373.1282) with the aid of ^{13}C NMR spectroscopic data. The IR absorption at 1758 cm^{-1} indicated the presence of a γ-lactone moiety. The ^1H

Received: June 23, 2015
Published: July 17, 2015

DOI: 10.1021/acs.orglett.5b01806
Org. Lett. 2015, 17, 3834−3837

NMR spectrum displayed resonances for a double bond at δ_H 7.49 (d, J = 2.1 Hz, H-16) and 6.78 (d, J = 2.1 Hz, H-15), an isolated olefinic proton at δ_H 6.52 (s, H-11), a methoxyl at δ_H 3.87 (s, OCH_3), and a methyl at δ_H 1.38 (s, CH_3-18) (Table 1).

Table 1. ^{1}H (400 MHz) Spectroscopic Data for 1 and 2

	1[a]	2[b]
no.	δ_H (J in Hz)	δ_H (J in Hz)
1	2.45 dd (3.2, 13.6)	2.69 d (11.4)
	2.34 ddd (3.4, 9.8, 13.6)	2.49 dd (2.0, 11.4)
2	4.25 dd (3.2, 9.8)	–
5	2.21 dd (2.0, 8.0)	2.57 d (5.8)
6	5.07 ddd (5.6, 8.0, 9.7)	5.06 ddd (3.4, 5.8, 9.4)
7	4.08 dd (9.7, 17.5)	4.08 dd (9.4, 18.4)
	2.92 dd (5.6, 17.5)	3.12 dd (3.4, 18.4)
11	6.52 s	6.41 s
15	6.78 d (2.1)	6.85 d (2.2)
16	7.49 d (2.1)	7.54 d (2.2)
18	1.38 s	1.40 s
20	4.06 dd (3.4, 9.8)	4.57 dd (2.0, 12.5)
	3.80 dd (2.0, 9.7)	4.08 d (12.5)
OCH_3	3.87 s	3.93 s

[a]Data acquired in $CDCl_3$–CD_3OD, 95:5. [b]Data acquired in $CDCl_3$.

The ^{13}C and DEPT NMR spectra exhibited 20 carbon signals corresponding to a methyl, a methoxyl, three methylenes, six methines, a dioxygenated secondary carbon, two oxygenated tertiary carbons, a carbonyl carbon, and five quaternary carbons (Table 2). A comparison of the ^{13}C NMR data of **1** with those

Table 2. ^{13}C NMR (100 MHz) Spectroscopic Data for 1 and 2

	1[a]	2[b]		1[a]	2[b]
no.	δ_C, type		no.	δ_C, type	
1	40.6 CH_2	41.2 CH_2	11	100.1 CH	98.9 CH
2	65.5 CH	173.4 C	12	152.3 C	153.0 C
3	96.6 C	80.4 C	13	116.8 C	117.4 C
4	48.4 C	54.5 C	14	154.0 C	154.2 C
5	51.3 CH	59.1 CH	15	104.3 CH	104.5 CH
6	74.4 CH	72.6 CH	16	144.0 CH	144.1 CH
7	25.4 CH_2	26.1 CH_2	18	19.3 CH_3	17.2 CH_3
8	109.4 C	110.4 C	19	179.5 C	178.7 C
9	133.4 C	134.3 C	20	70.5 CH_2	74.4 CH_2
10	35.8 C	46.3 C	OCH_3	55.6 CH_3	55.8 CH_3

[a]Data acquired in $CDCl_3$–CD_3OD, 95:5. [b]Data acquired in $CDCl_3$.

of icacinlactone B[10] revealed close similarities, except for C-1 and C-2. Compound **1** was bearing an additional hydroxyl group at C-2.

The structure of **1** was unambiguously confirmed by detailed interpretation of 2D NMR data (^{1}H–^{1}H COSY, HSQC, and HMBC) (Figures 1 and 2). The ^{1}H–^{1}H COSY spectrum displayed three coupled spin systems of H-1 (δ_H 2.45, 2.34)/H-2 (δ_H 4.25), H-5 (δ_H 2.21)/H-6 (δ_H 5.07)/H-7 (δ_H 4.08, 2.92), and H-15/H-16. The H-20b (δ_H 3.80) exhibited HMBC correlations with C-1 (δ_C 40.6), C-3 (δ_C 96.6), C-5 (δ_C 51.3), and C-10 (δ_C 35.8), allowing the establishment of connectivity between C-1 and C-5 via C-10, as well as the proposal of a 3,20-epoxy bridge. Indeed, the chemical shift of C-3 (δ_C 96.6) was in agreement with a hemiketal structure. The HMBC

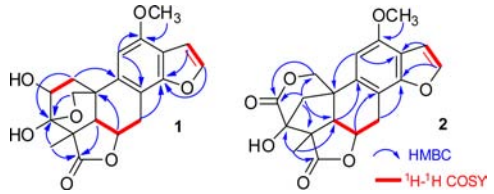


Figure 2. ^{1}H–^{1}H COSY and selective HMBC correlations of **1** and **2**.

correlation between H-1b (δ_H 2.34) and C-3 further confirmed the assignment of C-3. In the HMBC spectrum, CH_3-18 (δ_H 1.38) correlated with C-3, C-4, C-5, and C-19 (δ_C 179.5). On the other hand, the benzofuran part of the molecule was confirmed by the observation of the following HMBC cross signals: H-11 with C-8 (δ_C 109.4)/C-12 (δ_C 152.3)/C-13 (δ_C 116.8), both H-15 and H-16 with C-13 and C-14 (δ_C 154.0). Long-range correlations between H-11 and C-10 (δ_C 35.8), between OCH_3 and C-12, and between H-7 and C-14 were also detected.

The relative configuration of **1** could be proposed on the basis of NOESY correlations (Figure 3). Finally, crystals of **1**

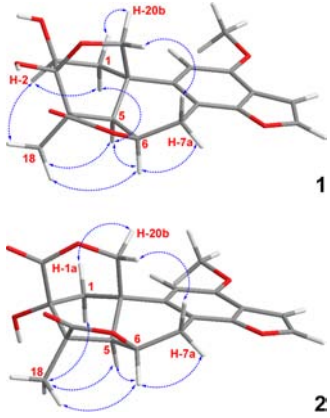


Figure 3. Selective NOESY correlations of **1** and **2**.

were available from a mixture of MeOH and EtOAc for X-ray diffraction analysis assisted by a CCD area detector using a Cu Kα X-ray source. The absolute configuration of **1** was thus determined to be 2*S*, 3*R*, 4*R*, 5*R*, 6*R*, and 10*R* (Figure 4). Furthermore, the electronic circular dichroism (ECD) spectra calculations for both **1** and its enantiomer were carried out using the time-dependent density functional theory (TDDFT) method. The calculated ECD for **1** matched its experimental data, showing similar Cotton effects (Figure 5).

Icacintrichantholide (**2**) was obtained as a colorless amorphous powder. The HRESIMS showed a quasi-molecular ion peak at m/z 393.0937 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_7Na$, 393.0945), suggesting a molecular formula $C_{20}H_{18}O_7$ with 12 indices of hydrogen deficiency. The IR absorption at 1769 cm^{-1} suggested the presence of lactone moieties. A comparison of ^{13}C NMR data between **1** and **2** revealed similarities but showed major differences in the structure of the A-ring (Table 2). As in the case of **1**, the assignments from C-4 to C-20 were achieved on the basis of the ^{1}H–^{1}H COSY, HSQC, and HMBC (Tables 1, 2; Figures 1, 2). The observation of HMBC correlations between CH_3-18 (δ_H 1.40) and C-4 (δ_C 54.5), C-5 (δ_C 59.1), C-19 (δ_C 178.7), and a carbon at δ_C 80.4 led to the assignment of the last signal to C-3. The presence of a 2,20-lactone and a five-membered A-ring were revealed by the

DOI: 10.1021/acs.orglett.5b01806
Org. Lett. 2015, 17, 3834–3837

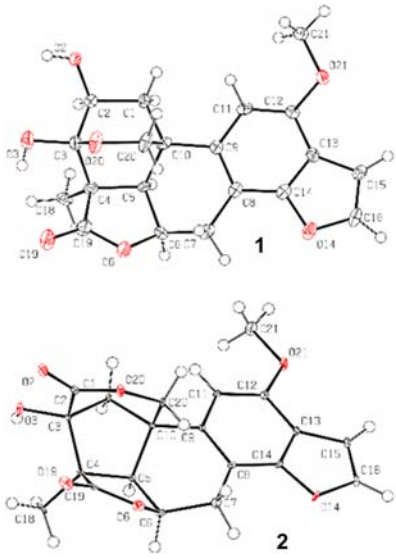


Figure 4. ORTEP representation of **1** and **2**.

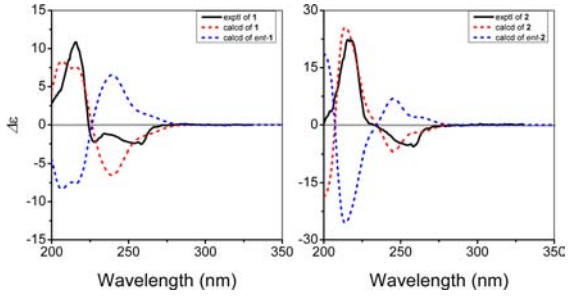


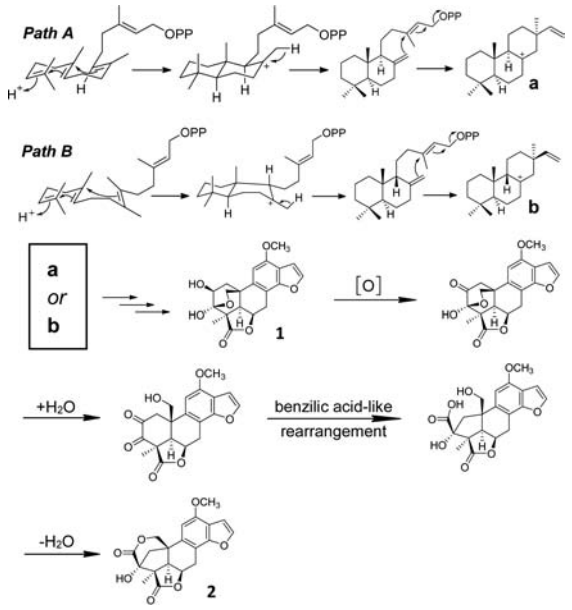
Figure 5. Experimental ECD spectra for **1** and **2**, and the calculated ECD spectra for **1**, **2**, and their enantiomers.

following HMBC correlations: H-1a (δ_H 2.69) with C-2 (δ_C 173.4), C-3 (δ_C 80.4), C-4, C-5, C-10 (δ_C 46.3), and C-20 (δ_C 74.4); H-20a (δ_H 4.57) with C-2. Compound **2** was thus elucidated to be a new diterpene possessing an unprecedented five-membered A-ring. The NOESY experiment (Figure 3) revealed the α-orientation of H-5, H-6, and 4-CH_3, as well as the β-orientation of 2,20-lactone, which were further confirmed by single-crystal X-ray diffraction (Figure 4).

To determine the absolute configuration of **2**, ECD spectra for (3*S*,4*R*,5*R*,6*R*,10*R*)-**2** and its enantiomer were calculated using the TDDFT method (see the Supporting Information). The experimental CD spectrum of **2** matched well with the calculated ECD of (3*S*,4*R*,5*R*,6*R*,10*R*)-**2** and was opposite to that of its enantiomer (Figure 5). The absolute configuration of **2** was thus determined to be 3*S*,4*R*,5*R*,6*R*,10*R*.

It is noteworthy that 17-norpimarane diterpene is a small class of natural products.[7,9−11] Compound **1** is proposed to be the biosynthetic precursor of **2**, and a plausible biogenetic pathway is shown in Scheme 1. In theory, there might be two possible biogenetic pathways for **1** and **2**, i.e. the pimarane (Path A) or (9βH)-pimarane pathway (Path B) (Scheme 1).[12] Since all diterpene structures isolated from *Icacina* plants so far bear a 9βH (if any), we prefer the (9βH)-pimarane pathway (Path B). It is suggested that the oxidation of 2-OH in **1** is followed by the loss of H_2O to form a 2,3-diketo intermediate. Subsequently, an enzymatic benzilic acid-like rearrangement would lead to the formation of a five-membered A-ring, followed by lactonization to afford **2**.[13,14]

Scheme 1. Plausible Biogenetic Pathway for 1 and 2



The diterpene class of compounds possesses not only a great variety of chemical structures but also a wide range of biological properties. Some have shown antiherpes virus (anti-HSV) activity.[15−17] However, when evaluated for in vitro antiviral activity, neither compound **1** nor **2** displayed significant activity against HSV-1, HSV-2, and the Epstein−Barr virus at concentrations up to 50 μM.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, 1D and 2D NMR, HRESIMS, UV, IR, ECD spectra and calculations of compounds **1** and **2**, and their X-ray crystal data (CIF). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01806.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mingz@uic.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.M.O. acknowledges the Institute of International Education of the United States, Department of State's Bureau for Education and Cultural Affairs for an award from the Fulbright Junior Scholar Development Exchange Program, Grantee ID No. 15120356, to conduct research at the UIC.

■ REFERENCES

(1) Asuzu, I. U.; Abubakar, I. I. *Phytother. Res.* **1995**, *9*, 21−25.
(2) Asuzu, I. U.; Ugwueze, E. E. *J. Ethnopharmacol.* **1990**, *28*, 151−156.
(3) Onakpa, M. M.; Asuzu, I. U. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 628−633.
(4) Timothy, O.; Idu, M. *Int. J. Med. Arom. Plants* **2011**, *1*, 184−188.
(5) Asuzu, I. U.; Egwu, O. K. *Phytomedicine* **1998**, *5*, 35−39.
(6) On'okoko, P.; Vanhaelen, M.; Vanhaelen-Fastre, R.; Declercq, J. P.; Van Meerssche, M. *Tetrahedron* **1985**, *41*, 745−748.

DOI: 10.1021/acs.orglett.5b01806
Org. Lett. 2015, 17, 3834−3837

(7) On'okoko, P.; Vanhaelen, M.; Vanhaelen-Fastre, R.; Declercq, J. P.; Van Meerssche, M. *Phytochemistry* **1985**, *24*, 2452−2453.
(8) On'okoko, P.; Vanhaelen, M. *Phytochemistry* **1980**, *19*, 303.
(9) Onakpa, M. M.; Zhao, M.; Goedecke, T.; Chen, W.-L.; Che, C. T.; Santarsiero, B. D.; Swanson, S. M.; Asuzu, I. U. *Chem. Biodiversity* **2014**, *11*, 1914−1922.
(10) Zhao, M.; Onakpa, M. M.; Chen, W.-L.; Santarsiero, B. D.; Swanson, S. M.; Burdette, J. E.; Asuzu, I. U.; Che, C.-T. *J. Nat. Prod.* **2015**, *78*, 789−796.
(11) Zoghbi, M. D. G. B.; Roque, N. F.; Gottlieb, H. E. *Phytochemistry* **1981**, *20*, 1669−1673.
(12) MacMillan, J.; Beale, M. H. In *Comprehensive Natural Products Chemistry* (Vol. 2); Cane, D. E., Ed.; Pergamon Press: 2000; pp 217−241.
(13) Grieco, P. A.; Speake, J. D. *J. Org. Chem.* **1998**, *63*, 5929−5936.
(14) Shudo, K.; Natsume, M.; Okamoto, T. *Chem. Pharm. Bull.* **1966**, *14*, 311−313.
(15) Betancur-Galvis, L.; Zuluaga, C.; Arno, M.; Gonzalez, M. A.; Zaragoza, R. J. *J. Nat. Prod.* **2001**, *64*, 1318−1321.
(16) Mucsi, I.; Molnar, J.; Hohmann, J.; Redei, D. *Planta Med.* **2001**, *67*, 672−674.
(17) Abrantes, J. L.; Barbosa, J.; Cavalcanti, D.; Pereira, R. C.; Fontes, C. F. L.; Teixeira, V. L.; Souza, T. M.; Paixao, I. C. P. *Planta Med.* **2010**, *76*, 339−344.

DOI: 10.1021/acs.orglett.5b01806
Org. Lett. 2015, 17, 3834−3837