Organic LETTERS

Letter

pubs.acs.org/OrgLett

Arboridinine, a Pentacyclic Indole Alkaloid with a New Cage Carbon–Nitrogen Skeleton Derived from a Pericine Precursor

Suet-Pick Wong,[†] Chew-Yan Gan,[‡] Kuan-Hon Lim,[§] Kang-Nee Ting,[∥] Yun-Yee Low,[†] and Toh-Seok Kam*[,†]

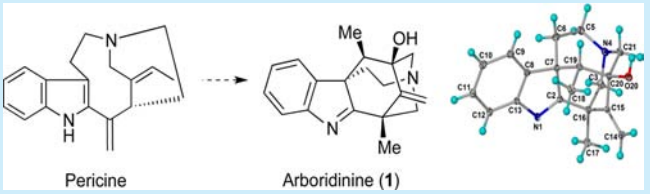
[†]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

[‡]University Malaysia of Computer Science and Engineering, Jalan Alamanda 2, Precint 1, 62000 Putrajaya, Malaysia

[§]School of Pharmacy and [∥]Department of Biomedical Sciences, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

Supporting Information

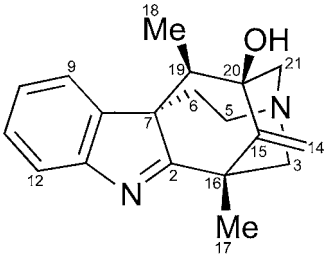
ABSTRACT: A new monoterpene indole alkaloid characterized by an unprecedented pentacyclic cage skeleton, arboridinine (**1**), was isolated from a Malaysian *Kopsia* species. The structure and absolute configuration of the alkaloid were determined based on NMR, MS, and X-ray diffraction analysis. A possible biogenetic pathway from a pericine precursor is presented.



Plants of the genus *Kopsia* (Apocynaceae) are distributed mainly over Southeast Asia, India, China, and Australia, with the majority of the species concentrated in Southeast Asia. Of these, about 16 species occur in Malaysia.[1,2] Plants belonging to this genus are rich in indole alkaloids, and the Malaysian representatives in particular have proven to be prodigious sources of novel alkaloids with unusual or intriguing carbon skeletons and interesting biological activity.[3] Some examples of such alkaloids characterized by challenging polycyclic skeletons, and previously isolated by us from *Kopsia*, include the pentacyclic, three nitrogen containing, arboflorine,[4] the caged arbophylline,[5] the antimelanin biosynthesis alkaloids, pauciflorines A and B,[6] and the curious tetracyclic alkaloid, mersicarpine, constituted from fusion of a dihydroindole, a seven-membered cyclic imine, and a δ-lactam moiety.[7] The latter two alkaloids especially have been the subject of a number of successful syntheses.[8,9] In continuation of our studies on the Malaysian members of this genus, we investigated the alkaloid content of *K. arborea* Blume and herein report the isolation and structure determination of a new pentacyclic monoterpenoid indole alkaloid, arboridinine (**1**), characterized by an unprecedented molecular skeleton and derived from a pericine precursor.

Arboridinine (**1**) was initially obtained as a colorless oil and subsequently crystallized from CH_2Cl_2/hexanes as colorless needles, mp 190–191 °C, $[\alpha]^{25}_D$ +205 ($CHCl_3$, *c* 0.23). The IR spectrum showed an OH absorption band at 3280 cm^{-1}, while the UV spectrum showed characteristic indolenine absorption maxima at 222 and 251 nm (log ε 3.71 and 3.28 respectively).[10a,b] The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 295, and HRESIMS measurements established the molecular formula as $C_{19}H_{22}N_2O$.[11]

The ^{1}H NMR data (Table 1) showed the presence of four aromatic resonances (δ 7.15–7.55), two singlets at δ 5.05 and 5.06, which are attributed to the methylene hydrogens of an exocyclic double bond, and two methyl groups at δ 0.39 (d, *J* = 7 Hz) and 1.51 (s). The ^{13}C NMR data (Table 1) showed a total of 19 carbon resonances, comprising two methyl, five methylene, five methine, two tertiary carbons linked to the indolic nitrogen (corresponding to C-2 and C-13), one hydroxyl-substituted tertiary carbon (δ 73.7), and four quaternary carbon atoms.



1

The resonance at δ 189.1 is typical of an imine carbon and is therefore assigned to C-2, while the other downfield carbon resonance observed at δ 153.3 was due to the quaternary carbon (C-15) of an exocyclic double bond, with the signal at 105.0 attributed to the corresponding geminal carbon (C-14). The carbon resonances of the indole unit can be readily assigned based on analogy with other alkaloids with an indolenine chromophore,[10] and these assignments were readily corroborated by NOE and 2D NMR data (Figures 1 and 2). It is to be noted that, of the two methyl resonances, the doublet at δ 0.39 was significantly shielded compared to the other, which

Received: June 17, 2015
Published: July 8, 2015

DOI: 10.1021/acs.orglett.5b01757
Org. Lett. 2015, 17, 3628–3631

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Arboridinine (1)[a]

H/C	δ_C	δ_H
2	189.1	–
3	62.3	3.09 d (14)
		3.11 d (14)
5α	53.0	3.20 ddd (15, 10, 5)
5β		3.46 ddd (15, 10, 5)
6α	34.1	1.57 ddd (15, 10, 5)
6β		2.32 ddd (15, 10, 5)
7	61.5	–
8	145.4	–
9	122.0	7.15 d (7.5)
10	125.6	7.18 t (7.5)
11	127.6	7.26 td (7.5, 1)
12	120.9	7.55 d (7.5)
13	152.8	–
14b	105.0	5.05 s
14a		5.06 s
15	153.3	–
16	49.3	–
17	18.1	1.51 s
18	12.6	0.39 d (7)
19	48.3	2.59 q (7)
20	73.7	–
21α	66.2	3.26 d (13)
21β		3.36 d (13)

[a]$CDCl_3$, 600 MHz.

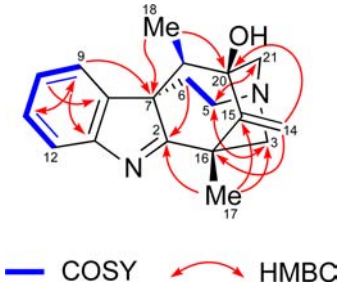


Figure 1. COSY and Selected HMBCs of **1**.

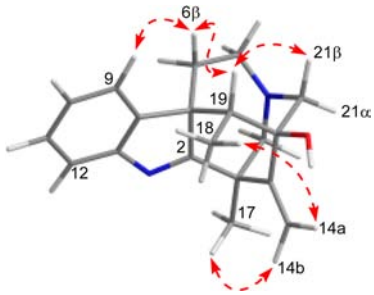


Figure 2. Selected NOEs of **1**.

was observed as a singlet at δ 1.51, an observation that would turn out to be of significance in the assignment of stereochemistry (vide infra).

The COSY spectrum (Figure 1) showed two partial structures, a NCH_2CH_2 and a CH_3CH fragment, in addition to the four contiguous aromatic hydrogens. Two isolated methylenes were observed, one at δ 3.09 and 3.11 (J = 14 Hz, δ_C 62.3), and the other at δ 3.26 and 3.36 (J = 13 Hz, δ_C 66.2). The ^{1}H and ^{13}C chemical shifts indicate that these methylenes are linked to N-4. Likewise, the methyl singlet at δ 1.51 (δ_C 18.1) indicated its attachment to a quaternary carbon. The remaining fragments include the exocyclic double bond and an OH attached to a tertiary carbon at δ_C 73.7.

The assignment of the NCH_2CH_2 fragment to C-5–C-6 was supported by the three-bond correlation from H-6 to C-2 in the HMBC spectrum (Figure 1) and by the observed NOE for H-9/H-6. The observed three-bond correlation from H-5 to C-21 (and H-21 to C-5), and from H-5 to C-3 (and H-3 to C-5), provided further support for the attachment of these isolated methylenes (C-3 and C-21) to N-4. The three-bond correlations from the methyl singlet at δ 1.51 to the indolenine C-2, C-3, and the olefinic C-15 indicated its attachment to the quaternary C-16, which is also consistent with the observed three-bond correlation from the exocyclic methylene hydrogens to C-16. The oxygenated C-20 is linked to the olefinic C-15 (from the observed three-bond correlation from H-14 to C-20), constituting part of a tertiary allylic alcohol fragment incorporating the C-14–C-15 exocyclic double bond. It remains only to insert the $CHCH_3$ fragment, and this is readily achieved from the observed three-bond correlations from the 18-methyl to C-7 and C-20, which require the methine C-19 to intervene between C-7 and C-20. This results in the formation of the remaining six-membered ring and completion of the assembly of this novel ring system.

The structure and relative configuration at the various stereogenic centers are entirely consistent with the NOE data (Figure 2). The previously noted unusual shielding of Me-18 is consistent with its β-disposition, which places it within the shielding zone of the indole moiety. The C-20 hydroxyl group and the methyl group attached to C-16 (Me-17) are both by necessity β-oriented as a consequence of the geometry imposed by the cage structure. These conclusions are supported by the observed NOEs. The reciprocal NOEs seen for Me-18/H-14a (δ 5.06) and Me-17/H-14b (δ 5.05) indicated their common β-orientation. NOEs were observed between H-9 and both H-6, with one (δ 2.32) stronger than the other (δ 1.57). Examination of models indicated attribution of the former to H-6β and the latter to H-6α. These NOEs also define the relative configuration of C-7. The NOEs observed for H-6β/H-19 and H-19/H-21 require these axially oriented hydrogens (H-6β, H-19, and H-21β) to be directed into the same face of the upper azepane ring, which is in turn consistent with the β-orientation of the C-20–OH group.

The structure of arboridinine (**1**), established by detailed examination of the spectroscopic data, was also verified by X-ray diffraction analysis, since suitable crystals were obtained.[12] Arboridinine (**1**) crystallized in the orthorhombic system with space group $P2_12_12_1$. The molecular structure was solved by using the program SHELXT and refined to a discrepancy index of 3.1%.[13] Although the structure contains no heavy atoms (>Si), and Mo Kα radiation was used as the X-ray source, the absolute configuration could be established using the X-ray diffraction methodology described recently by Escudero-Adán et al.[14] The Flack,[15] Hooft,[16] and Parsons[17] parameters were x = 0.0(2), y = 0.0(2), and z = 0.0(2), respectively. For the inverted structure, the Flack, Hooft, and Parsons parameters were x = 1.0(2), y = 1.0(2), and z = 1.0(2), respectively, from which it follows that the correct enantiomer is the one depicted in Figure 3.[14]

The structure of arboridinine represents a new and previously unknown skeleton of the monoterpenoid indoles.

DOI: 10.1021/acs.orglett.5b01757
Org. Lett. 2015, 17, 3628–3631

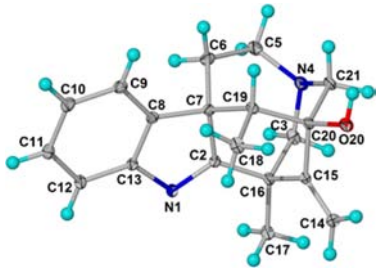
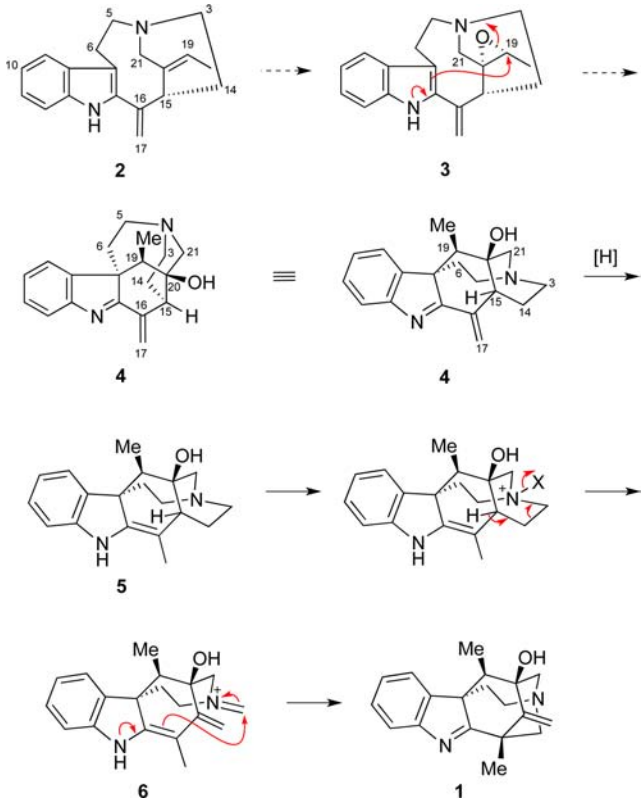


Figure 3. X-ray crystal structure of **1**.

The polycyclic ring system results in a novel as well as aesthetically pleasing caged architecture, the cage moiety circumscribed by two azepane, cyclohexyl, and piperidine rings. A plausible biogenetic pathway is shown in Scheme 1

Scheme 1. A Possible Pathway to 1 from 2



from a pericine precursor (**2**), which on oxidation of the ethylidene double bond furnishes the epoxide **3**, which was subsequently followed by nucleophilic attack by the electron-rich indole C-7 at C-19, forging the six-membered ring, incorporating the Me-18 substituent in the polycyclic imine alcohol **4**. Reduction (via conjugate addition of hydride) gives the enamine **5**. Installation of a suitable leaving group on N-4, followed in turn by a Grob-like fragmentation as shown, leads to the tetracyclic iminium intermediate **6**, which on subsequent intramolecular attack by the enamine gives the desired alkaloid, arboridinine (**1**). This pathway also leads to the correct absolute configuration of **1** as established by X-ray analysis (vide supra).

Compound **1** did not show any appreciable cytotoxicity against both drug-sensitive as well as vincristine-resistant KB cells, but showed a moderate concentration dependent relaxation effect on phenylephrine-induced contraction in isolated rat aortic rings (EC_{50} 4.98 μM, E_{max} 60.6 ± 7.8%; cf. isoprenaline, EC_{50} 0.08 μM, E_{max} 79.7 ± 4.2%).[18]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, 1D, 2D NMR, HRESIMS spectroscopic data, and X-ray crystallographic data (CIF) of **1**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01757.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: tskam@um.edu.my.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Malaya and MOHE Malaysia (HIR-005) for financial support.

■ DEDICATION

Dedicated to Dr. Swee-Hock Goh (former Chair of Organic Chemistry, University of Malaya, Kuala Lumpur, Malaysia), on the occasion of his 75th birthday.

■ REFERENCES

(1) Middleton, D. J. *Harvard Pap. Bot.* **2004**, *9*, 89−142.
(2) Middleton, D. J. In *Flora of Peninsular Malaysia*; Kiew, R., Chung, R. C. K., Saw, L. G., Soepadmo, E., Boyce, P. C., Eds.; Series II: Seed Plants, *Vol. II*; Forest Research Institute Malaysia, Kepong: Malaysia, 2011.
(3) (a) Kam, T. S.; Lim, K. H. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: Amsterdam, 2008; Vol. *66*, pp 1−111. (b) Kam, T. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: Amsterdam, 1999; Vol. *14*, pp 285−435.
(4) Lim, K. H.; Kam, T. S. *Org. Lett.* **2006**, *8*, 1733−1735.
(5) Lim, K. H.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 8653−8655.
(6) Kam, T. S.; Yoganathan, K.; Koyano, T.; Komiyama, K. *Tetrahedron Lett.* **1996**, *37*, 5765−5768.
(7) Kam, T. S.; Subramaniam, G.; Lim, K. H.; Choo, Y. M. *Tetrahedron Lett.* **2004**, *45*, 5995−5998.
(8) (a) Magnus, P.; Gazzard, L.; Hobson, L.; Payne, A. H.; Lynch, V. *Tetrahedron Lett.* **1999**, *40*, 5135−5138. (b) Magnus, P.; Hobson, L. A.; Westlund, N.; Lynch, V. *Tetrahedron Lett.* **2001**, *42*, 993−997. (c) Magnus, P.; Gazzard, L.; Hobson, L.; Payne, A. H.; Rainey, T. J.; Westlund, N.; Lynch, V. *Tetrahedron* **2002**, *58*, 3423−3443.
(9) (a) Xu, Z.; Wang, Q.; Zhu, J. *J. Am. Chem. Soc.* **2015**, *137*, 6712−6724. (b) Zhong, X.; Qi, S.; Li, Y.; Zhang, J.; Han, F. S. *Tetrahedron* **2015**, *71*, 3734−3740. (c) Lv, Z.; Li, Z.; Liang, G. *Org. Lett.* **2014**, *16*, 1653−1655. (d) Iwama, Y.; Okano, K.; Sugimoto, K.; Tokuyama, H. *Chem. - Eur. J.* **2013**, *19*, 9325−9334. (e) Iwama, Y.; Okano, K.; Sugimoto, K.; Tokuyama, H. *Org. Lett.* **2012**, *14*, 2320−2322. (f) Nakajima, R.; Ogino, T.; Yokoshima, S.; Fukuyama, T. *J. Am. Chem. Soc.* **2010**, *132*, 1236−1237. (g) Biechy, A.; Zard, S. Z. *Org. Lett.* **2009**, *11*, 2800−2803. (h) Magolan, J.; Carson, B. A.; Kerr, M. A. *Org. Lett.* **2008**, *10*, 1437−1440.
(10) (a) Lim, K. H.; Low, Y. Y.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 5037−5039. (b) Kam, T. S.; Nyeoh, K. T.; Sim, K. M.; Yoganathan, K. *Phytochemistry* **1997**, *45*, 1303−1305. (c) Verpoorte, R.; Van Beek, T. A.; Riegman, R. L. M.; Hylands, P. J.; Bisset, N. G. *Org. Magn. Reson.* **1984**, *22*, 328−335.
(11) HRESIMS found *m/z* 295.1817 (calcd for $C_{19}H_{22}N_2O$ + H, 295.1805).

DOI: 10.1021/acs.orglett.5b01757
Org. Lett. 2015, 17, 3628−3631

(12) The crystals of **1** are orthorhombic, belonging to space group $P2_12_12_1$, with a = 7.4987(2) Å, b = 12.2482(3) Å, c = 16.8063(3) Å, V = 1543.58(6) Å^3, Z = 4, T = 100(2) K, D_{calcd} = 1.267 g/cm^3, crystal size 0.45 × 0.38 × 0.25 mm^3, 36 099 reflections measured (5.88° ≤ 2θ ≤ 69.912°), 6449 unique (R_{int} = 0.0271, R_{sigma} = 0.0197) which were used in all calculations. The final R_1 value is 0.0314 [$I > 2\sigma(I)$] and wR_2 = 0.0864 (all data). The Flack (x), Hooft (y), and Parsons (z) parameters were determined using the software PLATON, and their values were found to be 0.0(2), 0.0(2), and 0.0(2), respectively. CCDC deposition number 1406618.

(13) Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Adv.* **2015**, *71*, 3−8.

(14) Escudero-Adán, E. C.; Benet-Buchholz, J.; Ballester, P. *Acta Crystallogr., Sect. B: Struct. Sci., Cryst. Eng. Mater.* **2014**, *70*, 660−668.

(15) (a) Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876−881. (b) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143−1148. (c) Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681−690.

(16) (a) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96−103. (b) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2010**, *43*, 665−668.

(17) (a) Parsons, S.; Flack, H. D.; Wagner, T. *Acta Crystallogr., Sect. B: Struct. Sci., Cryst. Eng. Mater.* **2013**, *69*, 249−259. (b) Parsons, S.; Wagner, T.; Presly, O.; Wood, P. A.; Cooper, R. I. *J. Appl. Crystallogr.* **2012**, *45*, 417−429.

(18) (a) Tsai, T. H.; Wang, G. J.; Lin, L. C. *J. Nat. Prod.* **2008**, *71*, 289−291. (b) Matsuo, H.; Okamoto, R.; Zaima, K.; Hirasawa, Y.; Ismail, I. S.; Lajis, N. H.; Morita, H. *Bioorg. Med. Chem.* **2011**, *19*, 4075−4079.

DOI: 10.1021/acs.orglett.5b01757
Org. Lett. 2015, 17, 3628−3631