Organic LETTERS

Letter

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Arborisidine and Arbornamine, Two Monoterpenoid Indole Alkaloids with New Polycyclic Carbon–Nitrogen Skeletons Derived from a Common Pericine Precursor

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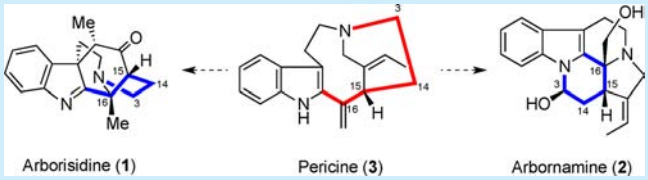
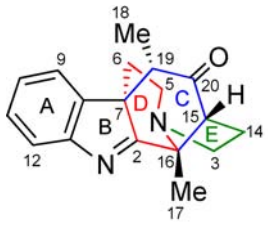
Supporting Information

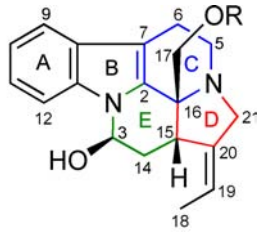
ABSTRACT: Two new monoterpene indole alkaloids, characterized by previously unencountered natural product skeletons, viz., arborisidine (**1**), incorporating indolizidine and cyclohexanone moieties fused to an indole unit, and arbornamine (**2**), incorporating an unprecedented 6/5/6/5/6 "arbornane" skeleton (distinct from the eburnan or tacaman skeleton), were isolated from a Malayan *Kopsia arborea*. The structures of the alkaloids were determined based on analysis of the NMR and MS data. Possible biogenetic pathways to these alkaloids from a common pericine precursor (**3**) are presented.



Plants of the genus *Kopsia* (Apocynaceae) are known to be rich repositories of alkaloids.[1] The genus comprises about 24 species distributed mainly over Southeast Asia, India, China, and Australia, with the majority of the species concentrated in Southeast Asia.[1,2] The Malaysian representatives of this genus (comprising about 16 species) have been particularly well-investigated and have resulted in the discovery of many new alkaloids with interesting structures,[3] a number of which have attracted the attention of synthetic chemists.[4] In a continuation of our studies on the Malayan *K. arborea* Blume,[3g] we report the isolation and structure determination of two new pentacyclic monoterpenoid indole alkaloids, arborisidine (**1**) and arbornamine (**2**), both characterized by unusual molecular skeletons and derived from a common pericine precursor (which also occurs in the plant).



1



2 R = H
9 R = Ac

Arborisidine (**1**) was isolated as a light yellowish oil, with $[\alpha]_D$ +12 (*c* 0.11, $CHCl_3$). The UV spectrum showed absorption maxima at 219 and 255 nm characteristic of an indolenine chromophore,[5] while the observed IR band at 1698 cm^{-1} indicated the presence of a carbonyl function. The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 281, and HRESIMS (high-resolution electrospray ionization mass spectrometry) measurements established the molecular formula as $C_{18}H_{20}N_2O$.[6]

The ^{1}H NMR data (Table 1) showed the presence of four aromatic resonances of an unsubstituted indole moiety (δ 7.26–7.80) and two methyl groups, one observed as a singlet at δ 1.63 and the other as a doublet at δ 1.45 (J = 7 Hz). The ^{13}C NMR data (Table 1) showed a total of 18 carbon resonances, comprising two methyls, four methylenes, six methines, three tertiary carbons linked to nitrogen (corresponding to C-2, C-13, and C-16), one ketocarbonyl, and two quaternary carbons. A deshielded resonance was observed at δ 186.2, which together with the absence of an indolic NH in the ^{1}H NMR spectrum and the characteristic UV absorptions, allowed assignment of this resonance to C-2 of an imine function. The carbon resonances of the indole unit can be readily assigned based on analogy with other alkaloids with an indolenine chromophore,[5] and these assignments were readily corroborated by NOE and 2D NMR data (Figures 1 and 2).

The COSY spectrum (Figure 1) showed the presence of the following partial structures: NCH_2CH_2, NCH_2CH_2CH, and CH_3CH. The NCH_2CH_2 fragment can be readily attributed to N–C-5–C-6 from the observed H-9/H-6 NOE and the three-bond correlations from H-3 to C-5 and from H-5 to C-16 in the HMBC spectrum (Figure 1). The observed H-3/C-5 correlation also indicated correspondence of the NCH_2CH_2CH fragment to N–C-3–C-14–C-15, while the observed H-5 to C-16 (δ 65.6) correlation is consistent with its (C-16) direct attachment to N-4. The methyl singlet (δ 1.63) is also attached to this carbon (C-16) from the three-bond correlations from this methyl to C-2 (δ

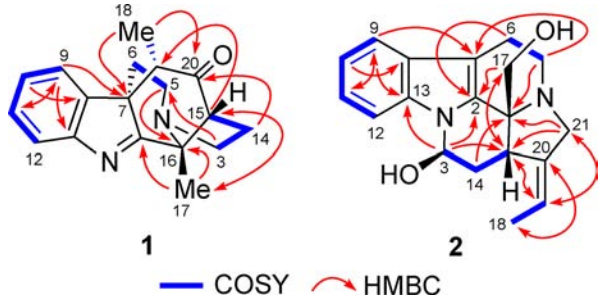
Received: February 19, 2016
Published: March 22, 2016

DOI: 10.1021/acs.orglett.6b00478
Org. Lett. 2016, 18, 1618–1621

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for 1, 2, and 9 in $CDCl_3$

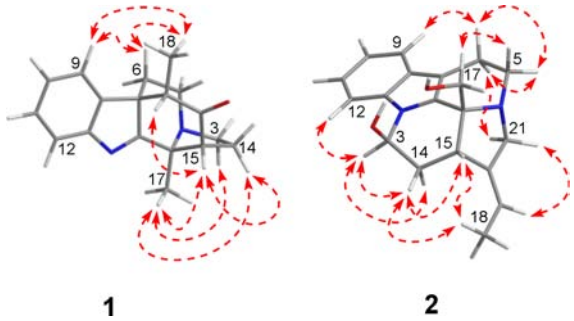
	1		2		9	
C/H	δ_C [a]	δ_H [b]	δ_C [c]	δ_H [b]	δ_C [a]	δ_H [b]
2	186.2		134.0		132.5	
3α	52.1	3.29 m	76.0	5.86 t (3)	76.1	5.85 t (3)
3β		2.87 m				
5α	48.0	2.85 m	41.4	3.21 dd (15, 6)	41.3	3.14 dd (14, 6)
5β		2.18 ddd (14, 12, 4)		3.43 m		3.32 m
6α	30.0	1.48 ddd (14, 12, 5)	16.6	2.91 ddd (16, 11, 6)	16.3	2.83 ddd (16, 11, 6)
6β		2.43 dt (14, 4)		2.64 dd (16, 6)		2.53 dd (16, 6)
7	56.3		107.8		109.6	
8	144.3		128.2		128.2	
9	122.9	7.45 m	118.8	7.52 d (8)	118.8	7.51 d (8)
10	125.5	7.26 m	120.3	7.15 t (8)	120.3	7.14 td (8, 1)
11	128.6	7.43 m	122.2	7.23 t (8)	122.3	7.22 td (8, 1)
12	121.7	7.80 d (8)	110.1	7.44 d (8)	110.0	7.42 d (8)
13	155.0		138.0		137.8	
14α	27.7	2.35 m	37.1	1.09 ddd (14, 10, 3)	36.5	1.10 ddd (14, 10, 3)
14β		2.86 m		2.68 ddd (14, 8, 3)		2.65 ddd (14, 8, 3)
15	57.6	2.67 d (9)	36.1	3.56 dd (10, 8)	35.4	3.36 dd (10, 8)
16	65.6		64.3		62.5	
17a	21.2	1.63 s	67.1	3.80 d (11)	68.0	4.44 br d (11)
17b				4.13 d (11)		4.50 d (11)
18	10.2	1.45 d (7)	14.1	1.65 d (6)	13.9	1.63 d (7)
19	52.2	2.08 q (7)	116.8	5.36 q (6)	116.2	5.34 qd (7, 2)
20	210.5		140.5		140.6	
21α			54.2	3.35 d (11)	54.4	3.24 br s
21β				3.30 d (11)		
CH_3CO_2–					21.1	2.06 s
CH_3CO_2–					171.5	

[a] 150 MHz. [b] 600 MHz. [c] 100 MHz.



1 2

Figure 1. COSY and selected HMBCs of 1 and 2.



1 2

Figure 2. Selected NOEs of 1 and 2.

186.2) and C-15 (δ 57.6) and a two-bond correlation from this methyl (Me-17) to C-16. These observations indicated direct bonding of the tertiary C-16 to C-2, N-4, C-15, and Me-17. The other methyl group (Me-18, δ 1.45, d, J = 7 Hz) is attached to a methine (C-19, δ_C 52.2; δ_H 2.08, q, J = 7 Hz), constituting the CH_3CH fragment observed in the COSY spectrum. The observed three-bond correlations from this methyl (Me-18) to the quaternary C-7 and the ketocarbonyl (C-20, δ_C 210.5) indicated the linking of C-19 to C-7 and C-20. The ketocarbonyl (C-20) is in turn linked to C-15 from the observed three-bond correlations from H-14 to the C-20 carbonyl and from H-15 to C-19. The combined 2D NMR (COSY, HMBC, NOESY) data are perfectly consistent with the structure for arborisidine, as shown in **1**.

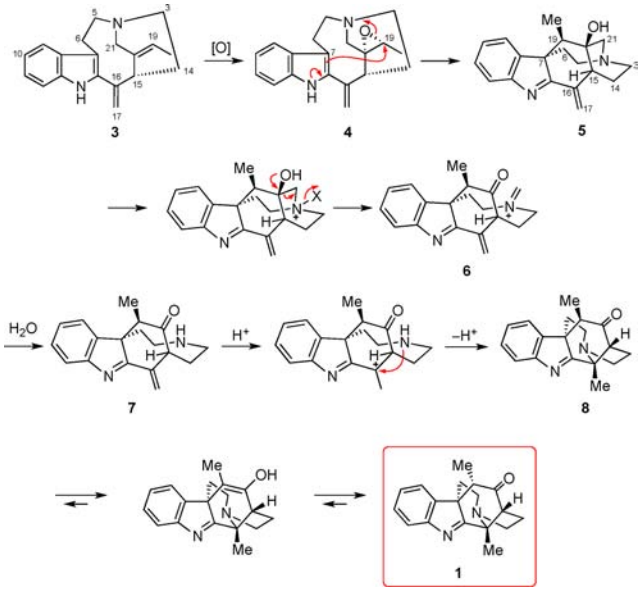
The relative configurations at the various stereogenic centers were deduced from the NOESY spectrum (Figure 2). *Cis*-fusion of the pyrrolidine ring at C-15 and C-16 requires H-15 and Me-17 to be *syn* to each other, with a β-oriented H-15 and an equatorially oriented Me-17 in the chair six-membered ring C. The observed H-15/H-19 reciprocal NOEs in turn require H-19 to be axially or β-oriented in the same cyclohexanone ring. The α-disposition of Me-18 is consistent with the observed Me-18/H-6β, H-9 NOEs. The equatorially oriented Me-17 showed NOEs with H-15, H-3α, and H-14α, which are consistent with the relative configuration at C-16. The resonance for H-15 was observed as a doublet with J = 9 Hz, as a result of H-14β and H-15 being orthogonal to each other, consistent with the geometry imposed by the polycyclic architecture of **1**.

Arborisidine (**1**) represents a new skeleton of the monoterpenoid indole alkaloids, characterized by an unusual and intriguing pentacyclic skeleton, incorporating indolizidine and cyclohexanone moieties fused to an indole unit at C-2 and C-7. A possible pathway to this alkaloid is from a pericine precursor **3**,[5a] which, on oxidation to the epoxide **4**, followed by intramolecular

DOI: 10.1021/acs.orglett.6b00478
Org. Lett. 2016, 18, 1618−1621

epoxide opening by the electron-rich indole C-7, furnishes the pentacyclic tertiary alcohol **5**. Installation of a suitable leaving group on N-4, followed by a Grob-like fragmentation, gives iminium ion **6**. Hydrolysis of the iminium ion with loss of formaldehyde leads to amino ketone **7**. Protonation of the exocyclic double bond followed by intramolecular nucleophilic capture of the resultant tertiary carbocation by N-4 leads to pentacyclic ketone **8**, possessing the essential ring system of arborisidine. The observed configuration at C-19 (C-19–αMe) requires a subsequent enol-mediated epimerization to the presumably thermodynamically more stable epimer **1** (Scheme 1).[7]

Scheme 1. Possible Pathway to 1 from 3



The second alkaloid arbornamine (**2**) was isolated as a light yellowish oil, with $[\alpha]_D$ −40 (*c* 0.07, $CHCl_3$). The UV spectrum showed characteristic indole absorption maxima at 225 and 271 nm, while the IR spectrum showed a broad band at 3324 cm^{-1} due to the presence of OH group(s). The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 311, and HRESIMS measurements established the molecular formula as $C_{19}H_{22}N_2O_2$.[8]

The ^{1}H NMR data (Table 1) showed the presence of four aromatic resonances of an unsubstituted indole moiety (δ 7.15–7.52), an ethylidene side chain (δ 5.36, q; 1.65, d; J = 6 Hz), an isolated aminomethylene (δ_H 3.30, 3.35; d, J = 11 Hz; δ_C 54.2), and an isolated oxymethylene (δ_H 3.80, 4.13; d, J = 11 Hz; δ_C 67.1). The former methylene (δ_C 54.2) must be branched from N-4, while the oxymethylene (δ_C 67.1) constitutes part of a hydroxymethyl group. The presence of the latter was supported by formation of an *O*-acetyl derivative **9** on acetylation (Ac_2O, py) of **2**.[9] The ^{13}C NMR spectrum (Table 1) accounted for all 19 carbon resonances comprising one methyl, five methylenes, seven methines, three tertiary carbons linked to nitrogen (corresponding to C-2, C-13 linked to the indolic N-1, and C-16 linked to N-4), and three quarternary carbon atoms. In addition to the eight carbon resonances, which are typical of the indole moiety, two olefinic resonances were observed at δ 116.8 and 140.5, which were readily assigned to the olefinic carbons (C-19 and C-20, respectively) of an ethylidene side chain from the corresponding ^{1}H resonances observed at δ 5.36 (H-19) and 1.65 (H-18). Furthermore, the notably deshielded methine resonance at δ 76.0 suggested connection of this methine to both an oxygen and a nitrogen atom.

The COSY data (Figure 1) showed, in addition to the aromatic hydrogens and the ethylidene side chain, the presence of two other partial structures, NCH_2CH_2 and $CHCH_2CH$, and the hydroxymethyl and isolated aminomethine fragments mentioned earlier. The NCH_2CH_2 fragment can be assigned to N–C-5–C-6 from the observed three-bond correlations from H-5 to C-7 and from H-6 to C-2 in the HMBC spectrum (Figure 1). The three-bond correlations from H-5 and the aminomethylene (H-21) to the tertiary carbon at δ_C 64.3 indicated that this carbon (C-16) is linked to N-4. Three-bond correlations from H-21 to the olefinic methine at δ_C 116.8 (C-19) and from Me-18 (δ_H 1.65) to the quaternary olefinic carbon at δ_C 140.5 (C-20) indicated connection of this carbon (C-20) to C-21. The $CHCH_2CH$ fragment has one deshielded terminal methine (δ_C 76.0; δ_H 5.86) characteristic of a carbinol amine, such as those seen in eburnamine and isoeburnamine, and, as such, must correspond to a hydroxy-substituted methine (C-3) branched from the indolic nitrogen N-1.[1] This conclusion is supported by the observed three-bond correlations from H-3 to C-2 and C-13. Attachment of the other terminal methine (δ 36.1, C-15) of this fragment is to C-16 (δ 64.3) and the quaternary olefinic C-20 (δ 140.5), from the observed three-bond correlations from H-14 to C-16 and from H-15 to C-19. Finally, the remaining hydroxymethyl group is substituted at C-16, from the correlation of the oxymethylene hydrogens (H-17) to C-2 and C-15. The resulting structure, as shown in **2**, is entirely consistent with the full HMBC (Figure 1) and NOE data (Figure 2) of **2** and the NMR data of the *O*-acetyl derivative **9**.

The relative configuration at the various stereogenic centers and the preferred conformation adopted by the molecule are fully consistent with the vicinal coupling and the NOE data (Figure 2). The geometry of the 19,20-double bond is *E* from the observed H-19/H-21β and Me-18/H-14β NOEs. The assignment of H-9 and H-12 follows from the respective H-9/H-6β and H-12/H-3 NOEs. H-6α was seen as a ddd with J = 16, 11, and 6 Hz. The J_{5-6} coupling of 11 Hz requires H-5β to be *trans*-diaxial with H-6α. The β-oriented (axially disposed) H-5 showed NOE with the oxymethylene H-17, requiring H-5β and the hydroxymethyl group to be *syn* to each other. The hydroxymethyl group must be similarly β-oriented. These observations indicated an approximate chair conformation adopted by ring C. The six-membered ring E incorporating a hydroxymethine linked to the indolic nitrogen bears a similarity to the situation in the eburnane compounds, notwithstanding possible modification of the ring conformation due to fusion of the adjacent five-membered pyrrolidine ring (instead of a six-membered piperidine ring in eburnane compounds) and the presence of a relatively bulkier hydroxymethyl substituent on C-16 instead of H. With an equatorially or β-oriented C-3–OH in a chair six-membered ring E,[10] H-3 should be observed as a dd with 9 and 5 Hz coupling, with the larger coupling due to a *trans*-diaxially disposed H-3 and H-14β, by analogy with the eburnane compounds.[1,11] However, H-3 was observed as a triplet with J = 3 Hz, which is inconsistent with such a conformation. The observed NOEs and coupling behavior of H-3, H-14, and H-15 become intelligible and perfectly consistent if the conformation adopted is that of a twist-boat instead of chair (to presumably avoid unfavorable hydroxymethyl/H-14β 1,3-diaxial interactions). Thus, in the twist-boat conformation, with C-3–βOH, the observed multiplicities and coupling constants and those calculated from the dihedral angles are as follows: $J_{14\beta\text{-}15(obsd)}$ = 8

DOI: 10.1021/acs.orglett.6b00478
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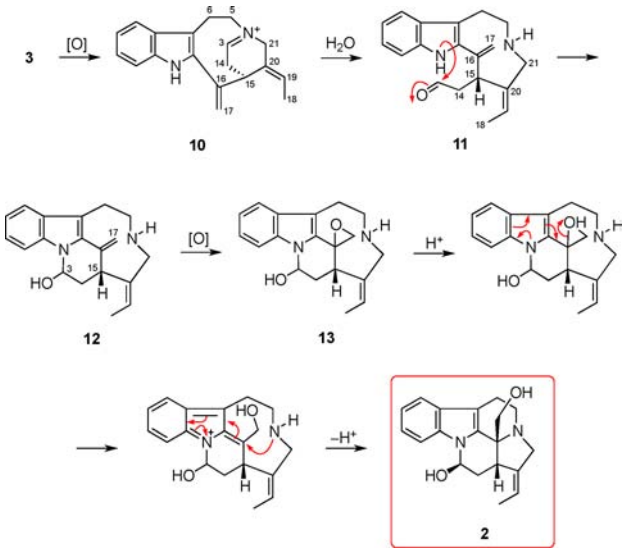
Hz, $J_{14\beta\text{-}15(calcd)}$ = 7.0 Hz; $J_{14\alpha\text{-}15(obsd)}$ = 10 Hz, $J_{14\alpha\text{-}15(calcd)}$ = 10.0 Hz; $J_{14\beta\text{-}3(obsd)}$ = 3 Hz, $J_{14\beta\text{-}3(calcd)}$ = 2.2 Hz; $J_{14\alpha\text{-}3(obsd)}$ = 3 Hz, $J_{14\alpha\text{-}3(calcd)}$ = 3.3 Hz, where there is excellent agreement between experimental and calculated J values.[12,13] The proposed structure and conformation are also consistent with the results from DFT calculations [B3LYP/6-31G(d)], where the twist-boat conformer is estimated to be more stable compared to the chair conformer by about 9 kJ mol^{-1}.

Arbornamine represents a pentacyclic alkaloid with an unprecedented 6/5/6/5/6 "arbornane" skeleton, which is distinct from the eburnane or tacaman skeleton. A possible biogenetic pathway to **2** is shown in Scheme 2 from the same pericine precursor **3** as in the case of **1**. Oxidation of **3** leads to the iminium ion **10**, which on hydrolytic cleavage gives aldehyde **11**. Intramolecular nucleophilic addition by the indolic nitrogen forges the tetracyclic carbinol amine **12**. Oxidation of the exocyclic double bond leads to epoxide **13**, which on subsequent lone-pair (N-1)-assisted epoxide ring opening, followed by secondary amine (N-4) conjugate addition, results in the hydroxymethyl-substituted pentacycle **2**.

Scheme 2. Possible Pathway to 2 from 3



Compounds **1** and **2** did not show any appreciable cytotoxicity when tested against KB, PC-3, A549, HCT116, and HT-29 cells.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00478.

Experimental procedures, 1D, 2D NMR, and HRESIMS data of compounds **1**, **2**, and **9**, ECD data for **1**, calculated free energies, and Cartesian coordinates for **2** (chair and twist-boat conformers) (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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

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(6) HRESIMS found m/z 281.1649 (calcd for $C_{18}H_{20}N_2O$ + H, 281.1648).

(7) This pathway also leads to an absolute configuration for **1** (7*R*,15*R*,16*S*,19*S*) that is the same as that obtained from analysis of the ECD data (see Supporting Information).

(8) HRESIMS found m/z 311.1760 (calcd for $C_{19}H_{22}N_2O_2$ + H, 311.1754).

(9) Compound **9**: $[\alpha]_D$ −14 (*c* 0.08, $CHCl_3$); HRESIMS found m/z 353.18704 $[M + H]^+$ (calcd for $C_{21}H_{24}N_2O_3$ + H, 353.18652); UV (EtOH) λ_{max} (log ε) 226 (4.43), 275 (3.89) nm; ^{1}H and ^{13}C NMR, see Table 1.

(10) Alternative C-3 configuration with an α-oriented OH for both chair or twist-boat conformations is incompatible with the observed vicinal coupling and NOE data.

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(12) Dihedral angles were obtained from DFT geometry-optimized structures and the coupling constants calculated using the Karplus equation (see ref 13).

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DOI: 10.1021/acs.orglett.6b00478
Org. Lett. 2016, 18, 1618–1621