Tetrahedron Letters 50 (2009) 1059–1061

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Leucolusine, a tetracyclic alkaloid with a novel ring system incorporating an oxindole moiety and fused piperidine-tetrahydrofuran rings

Chew-Yan Gan, Toh-Seok Kam *

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 31 October 2008
Revised 12 December 2008
Accepted 16 December 2008
Available online 24 December 2008

Keywords:
Indole alkaloids
NMR
Leuconotis
Apocynaceae

ABSTRACT

A tetracyclic ring-opened oxindole alkaloid, possessing an unprecedented ring system incorporating fused piperidine-tetrahydrofuran rings, has been isolated from the Malayan species, *Leuconotis griffithii*. The structure was established by analysis of the spectroscopic data and a possible biogenetic pathway from an *Aspidosperma* precursor is presented.

The genus *Leuconotis* (Apocynaceae) comprises a small group of 10 species,[1] of which three (*L. griffithii*, *L. maingayi*, and *L. eugenifolia*) are found in Peninsular Malaysia.[2,3] These plants are typically woody climbers, and previous studies of the Malayan *L. griffithii* and *L. eugenifolia* had provided in addition to the ring-opened alkaloids, leuconolam and rhazinilam and their derivatives, various strychnan, kopsan, and eburnan derivatives,[4,5] while several yohimbines and the pentacyclic diazaspiro alkaloid, leuconoxine, were subsequently reported from *L. eugenifolia* occurring in Indonesia.[6] The alkaloidal composition bears a striking similarity to plants of the genus *Kopsia*.[7] In continuation of our studies of biologically active alkaloids from Malaysian *Apocynaceae*,[7–23] we report the isolation of a new indole alkaloid, leucolusine (**1**), from the stem-bark extract of *L. griffithii*. Compound **1** is characterized by an unusual tetracyclic ring system, incorporating an oxindole moiety and fused piperidine-tetrahydrofuran rings.

Leucolusine (**1**) was isolated in a very small amount as a light yellowish oil following repeated chromatographic fractionation of the basic fraction from the EtOH extract; $[\alpha]_D$ +55 ($CHCl_3$, *c* 0.12). The UV spectrum (EtOH) was characteristic of an unsubstituted oxindole chromophore showing absorption maxima at 206, 252, and 288 nm,[24] while the IR spectrum showed bands at 3406, 3249, and 1716 cm^{-1}, suggesting the presence of OH, NH, and lactam functionalities, respectively. The EIMS of 1 showed a molecular ion peak at *m/z* 330, and HREIMS measurements established the molecular formula as $C_{19}H_{26}N_2O_3$, requiring eight degrees of unsaturation.[25] Strong fragment peaks were observed at *m/z* 182 (47%) and 168 (100%), which though initially puzzling, became intelligible after eventual unravelling of the overall structure (vide infra). The ^{1}H NMR spectrum (Table 1) showed the presence of four aromatic hydrogens (δ 6.74–7.37), an indolic NH (δ 7.63), a broad downfield signal due to OH (δ 8.13), a relatively downfield one-H singlet due to H-21 (δ 4.29), and an ethyl side chain (δ 0.84; 1.37, 1.55). The ^{13}C NMR spectrum (Table 1) showed a total of 19 carbon resonances (one methyl, eight methylenes, five methines, and five quaternary carbons) in agreement with the molecular formula. The six aromatic carbon resonances can be readily assigned from consideration of their characteristic shifts as well as from HMBC and NOE data (Figs. 1 and 2, respectively).

The observed NOE between the indolic NH and the aromatic doublet of doublets at δ 6.74 allowed assignment of this resonance to H-12 and the lower field doublet of doublets at δ 7.37 to H-9. The three-bond correlations from H-9 and NH to the downfield quaternary signal at δ 77.3 indicated that this signal was due to C-7, which, from its downfield shift, suggested some form of oxygenation. The C-7 substituent was deduced to be an OH from the IR, MS, and ^{1}H NMR data. Another downfield quaternary signal at δ 180.5 indicated the presence of an oxindole functionality, which received further support from the observed three-bond correlation from H-6 to the oxindole C-2. The COSY spectrum revealed three partial structures, viz., an $-NCH_2CH_2$ fragment corresponding to NC(5)–C(6), an OCH_2CH_2 fragment corresponding to OC(16)–C(17), and an $-NCH_2CH_2CH_2$ fragment corresponding to NC(3)–C(14)–C(15). The latter fragment was deduced with the aid of HMBC (three-bond correlations from H-3 and H-21 to C-15), as the H-15 resonances were partially overlapped with the H-14 and H-19 signals. The singlet observed at δ 97.7 was attributed

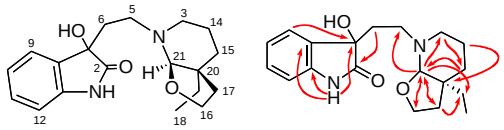
* Corresponding author. Tel.: +60 3 79674266; fax: +60 3 79674193.
E-mail address: tskam@um.edu.my (T.-S. Kam).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.12.077

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** in $CDCl_3$[a]

Position	δ_C	δ_H
2	180.5	—
3	44.6	2.69 (m)
3′		2.69 (m)
5	50.2	2.86 (dt, 13.5, 4 Hz)
5′		3.38 (ddd, 13.5, 11, 3 Hz)
6	32.2	1.65 (m)
6′		2.12 (ddd, 15, 11, 4 Hz)
7	77.3	—
8	132.3	—
9	124.1	7.37 (dd, 7.6, 1 Hz)
10	122.8	6.98 (td, 7.6, 1 Hz)
11	129.2	7.15 (td, 7.6, 1 Hz)
12	110.2	6.74 (dd, 7.6, 1 Hz)
13	140.0	—
14	21.2	1.53 (m)
14′		1.55 (m)
15	26.9	1.35 (m)
15′		1.56 (m)
16	64.1	3.83 (td, 9, 4 Hz)
16′		3.94 (dd, 16.6, 9 Hz)
17	35.1	1.60 (m)
17′		1.75 (m)
18	8.9	0.84 (t, 7.6 Hz)
19	27.0	1.37 (m)
19′		1.55 (m)
20	42.4	—
21	97.7	4.29 (s)
NH	—	7.63 (s)
OH	—	8.13 (br s)

[a] Assignments based on COSY, HMQC, and HMBC.



1

Figure 1. Selected HMBCs of **1**.

to the isolated methine corresponding to C-21, its downfield shift due to it being linked to both a nitrogen (N-4) and an oxygen atom.

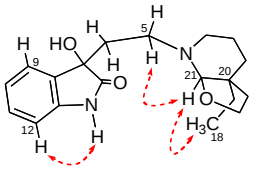
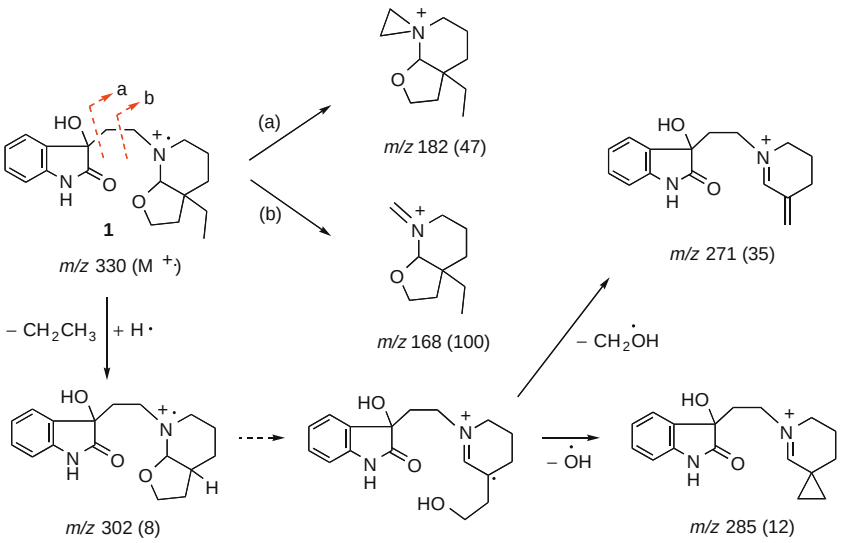


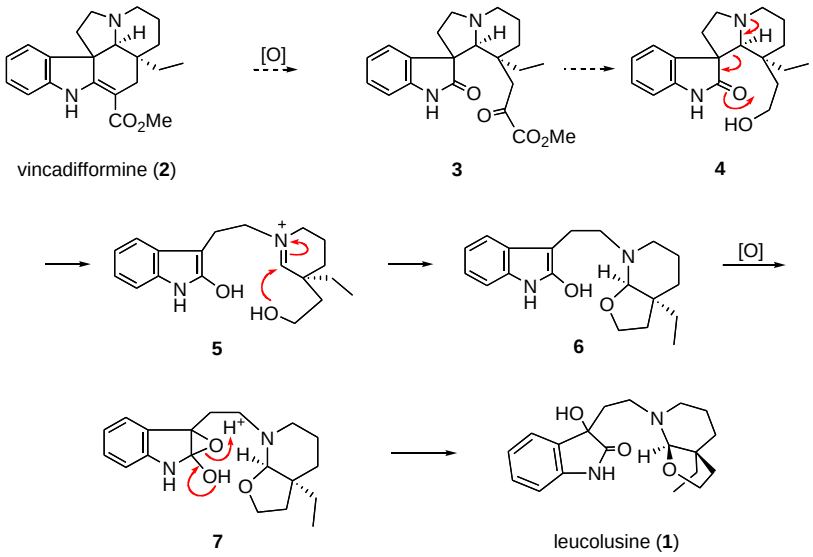
Figure 2. Selected NOEs of **1**.

The above data permitted assembly of the complete structure of leucolusine. The C-7 hydroxy-substituted oxindole chromophore is attached via C-7 to one end of the C(5)–C(6) fragment, the other end being attached to N-4. This was supported by the observed HMBC correlations from H-6 to C-2 and from H-21 to C-5. The N-4 atom forms part of an ethyl-substituted cis-fused piperidine-tetrahydrofuran ring (a 3a-ethyloctahydrofuro[2,3-*b*]pyridine unit) as indicated by the HMBC and NOE data. Thus, the three-bond correlations from H-21 to C-3 and from H-3 to C-21 indicated connection of C-21 to N-4, while the observed three-bond correlation from H-14 to C-20 indicated attachment of C-15 to the quaternary C-20. The observed H-15/C-21 and H-21/C-15 correlations indicated that C-21 and C-20 are contiguous forming part of the piperidine ring. The observed correlation from H-16 to C-21 and from H-17 to C-21 permitted assembly of the tetrahydrofuran ring, cis-fused at C-20 and C-21, while the observed correlations from H-18 to C-20 and from H-17, H-21 to C-19 indicated attachment of the ethyl side chain at the quaternary C-20. The cis-fusion of the piperidino-tetrahydrofuran rings was deduced from the observed NOE between H-18 and H-21, (Fig. 2) which also fixes the relative configurations at C-20 and C-21.

The structure thus unravelled is completely in agreement with the full HMBC and NOE data. The completed structure also permitted interpretation of the mass spectrum of leucolusine as shown in Scheme 1, in particular the origin of the strong fragments observed at *m/z* 168 and 182, due to scission of the C(5)–C(6) and C(6)–C(7) bonds, respectively. A possible origin of this unusual ring-opened alkaloid is from an *Aspidosperma* precursor such as vincadifformine[26] (**2**) (Scheme 2). Oxidative cleavage of **2** gives the oxindole-ketoester **3**, which on subsequent decarboxylation followed by reduction gives the alcohol **4**. A Grob-like, lone-pair assisted fragmentation of **4** leads to cleavage of the C(7)–C(21) bond, furnishing the iminium ion **5**, which on intramolecular trapping by



Scheme 1. Mass spectral fragmentation of **1**.



Scheme 2. A possible biogenetic pathway to **1.**

OH yields the tetracyclic intermediate **6**. Oxidation of **6** gives the epoxide **7**, which on subsequent oxidation at C-2 with concomitant epoxide ring-opening leads to leucolusine (**1**).[27] The proposed pathway leads to the relative configuration as shown in **1** although the available data do not allow assignment of the configuration at C-7.

Acknowledgment

We thank the University of Malaya and MOSTI, Malaysia (Science Fund) for financial support.

References and notes

1. Markgraf, F. *Blumea* **1971**, *19*, 155.
2. Whitmore, T. C. In *Tree Flora of Malaya*; Whitmore, T. C., Ed.; Academic Press: London, 1972; Vol. 2, pp 3–24.
3. Ridley, H. N. In *The Flora of the Malay Peninsula*; Reeve, L., Ed.; Academic Press: London, 1923; Vol. 2, pp 328–330.
4. Goh, S. H.; Chen, W.; Ali, A. R. M. *Tetrahedron Lett.* **1984**, *25*, 3483.
5. Goh, S. H.; Ali, A. R. M.; Wong, W. H. *Tetrahedron* **1989**, *45*, 7899.
6. Abe, F.; Yamauchi, T. *Phytochemistry* **1994**, *35*, 169.
7. Kam, T. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: Amsterdam, 1999; Vol. 14, pp 285–435.
8. Kam, T. S.; Choo, Y. M. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: Amsterdam, 2006; Vol. 63, pp 181–337.
9. Kam, T. S.; Tan, S. J.; Ng, S. W.; Komiyama, K. *Org. Lett.* **2008**, *10*, 3749.
10. Kam, T. S.; Lim, K. H. *Org. Lett.* **2006**, *8*, 1733.
11. Kam, T. S.; Lim, K. H. *Tetrahedron Lett.* **2007**, *48*, 1143.
12. Kam, T. S.; Lim, K. H. *Tetrahedron Lett.* **2006**, *47*, 8653.
13. Kam, T. S.; Subramaniam, G.; Choo, Y. M.; Hiraku, O.; Komiyama, K. *Tetrahedron* **2008**, *64*, 1397.
14. Kam, T. S.; Subramaniam, G. *Tetrahedron Lett.* **2004**, *45*, 3521.
15. Kam, T. S.; Subramaniam, G.; Lim, K. H.; Choo, Y. M. *Tetrahedron Lett.* **2004**, *45*, 5995.
16. Kam, T. S.; Choo, Y. M. *Tetrahedron Lett.* **2003**, *44*, 8787.
17. Kam, T. S.; Choo, Y. M. *Tetrahedron Lett.* **2003**, *44*, 1317.
18. Kam, T. S.; Choo, Y. M.; Komiyama, K. *Tetrahedron* **2004**, *60*, 3957.
19. Kam, T. S.; Sim, K. M.; Lim, T. M. *Tetrahedron Lett.* **2001**, *42*, 4721.
20. Kam, T. S.; Sim, K. M.; Lim, T. M. *Tetrahedron Lett.* **2000**, *41*, 2733.
21. Kam, T. S.; Sim, K. M.; Lim, T. M. *Tetrahedron Lett.* **1999**, *40*, 5409.
22. Kam, T. S.; Sim, K. M.; Koyano, T.; Toyoshima, M.; Hayashi, M.; Komiyama, K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1693.
23. Kam, T. S.; Pang, H. S.; Lim, T. M. *Org. Biomol. Chem.* **2003**, *1*, 1292.
24. Sharma, P.; Shirataki, Y.; Cordell, G. A. *Phytochemistry* **1988**, *27*, 3649.
25. Compound **1**, leucolusine, EIMS, *m/z* (rel. int.): 330 [M^+] (7), 302 (8), 285 (12), 271 (35), 182 (47), 168 (100), 154 (19), 140 (40). HREIMS found *m/z* 330.1942 (calcd for $C_{19}H_{26}N_2O_3$, 330.1943).
26. Gosset, J.; Men, J. L.; Janot, M. M. *Ann. Pharm. Fr.* **1962**, *20*, 448.
27. Leucolusine (**1**) did not show any appreciable cytotoxicity when tested against human KB cells (IC_{50} > 25 mg/mL).