Tetrahedron Letters 49 (2008) 5935–5938

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Two novel indole alkaloids, Kopsiyunnanines A and B, from a Yunnan *Kopsia*

Yuqiu Wu [a], Mariko Kitajima [a], Noriyuki Kogure [a], Rongping Zhang [b], Hiromitsu Takayama [a,*]

[a] Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
[b] Department of Pharmaceutical Sciences, Kunming Medical College, Kunming 650031, Yunnan Province, China

ARTICLE INFO

Article history:
Received 28 June 2008
Revised 25 July 2008
Accepted 28 July 2008
Available online 31 July 2008

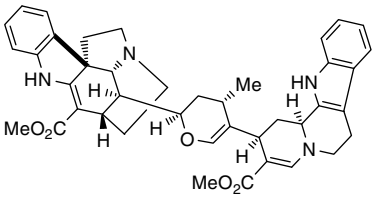
Keywords:
Apocynaceae
Kopsia
Indole alkaloids
NMR

ABSTRACT

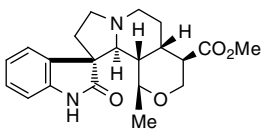
Two novel indole alkaloids having unusual skeletons were isolated from the aerial part of Yunnan *Kopsia arborea*. Kopsiyunnanine A (**1**) is a new class of bisindole alkaloid composed of vallesiachotamine (modified Corynanthe-type) and Aspidospermatan-type alkaloids. Kopsiyunnanine B (**2**) is a new Corynanthe-type oxindole alkaloid rearranged by D ring rotation.

The genus *Kopsia* consists of 23 species of evergreen trees and shrubs that are widely distributed over Southeast Asia.[1] Four species are found in China.[2] However, only *Kopsia officinalis* Tsiang et P. T. Li (it was altered to synonym of *Kopsia arborea* in the light of Dr. Midddleton's latest revision[1]), growing in Yunnan Province, Southwestern China, has been reported for its use in the treatment of rheumatoid arthritis, dropsy, and tonsillitis.[3] The chemical investigation of *Kopsia* has yielded many alkaloids with unusual or intriguing carbon skeletons and interesting biological activities.[4]

As part of serial investigations on novel and bioactive indole alkaloids,[5] phytochemical research on Yunnan *K. arborea* (*K. officinalis*) was carried out. Here we describe the isolation and structure elucidation of two novel indole alkaloids. Kopsiyunnanine A (**1**) is a new class of bisindole alkaloid composed of vallesiachotamine (modified Corynanthe-type) and Aspidospermatan-type alkaloids. Kopsiyunnanine B (**2**) is a new Corynanthe-type oxindole alkaloid rearranged by D ring rotation.



Kopsiyunnanine A (**1**)



Kopsiyunnanine B (**2**)

Kopsiyunnanine A (**1**)[6] was obtained as a yellowish amorphous solid. Its molecular formula was determined to be $C_{41}H_{44}N_4O_5$ based on HRFABMS (*m/z* 673.3359 $[M+H]^+$, calcd for 673.3390), which is indicative of a dimeric molecule composed of two monoterpenoid indole alkaloids. Characteristic absorptions at λ_{max} 224.5 and 291.5 nm in the UV spectrum, which are due to the β-*N*-acrylate chromophore, reminded us of the well-known alkaloid vallesiachotamine (**3**)[7] that coexists in the same plant. In addition, absorptions at λ_{max} 224.5, 291.5, and 330.0 nm suggested the presence of an anilinoacrylate chromophore in the dimer. In fact, indole alkaloids possessing the same chromophore, such as Aspidospermatan-type alkaloids, including tubotaiwine (**5**)[8] and condylocarpine,[9] were also isolated in our present research.

Careful comparison of the ^1H NMR and ^{13}C NMR spectra of **1** (Table 1) with those of vallesiachotamine (**3**) and Aspidospermatan-type alkaloids showed high resemblance, as anticipated. Besides the signals assigned to the four aromatic protons on A ring of the indole nucleus, the ^1H NMR and ^{13}C NMR spectra contained signals obviously attributed to vallesiachotamine (Unit A) with an indolic NH group (δ_H 9.00, br s), a C-3 methine group (δ_H 4.40, δ_C 47.7), a C-17 olefinic group (δ_H 7.60, δ_C 146.5), and a carbonyl ester group (δ_H 3.62, δ_C 50.7, δ_C 168.7), but no signals due to aldehyde proton and ethylidene side chain were present in **3**. Other signals also gave significant information implying the presence of an Aspidospermatan-type alkaloid (Unit B). The two downfield quaternary carbon signals at δ_C 96.2 and δ_C 168.8 were typical of C-16′ and C-2′, respectively. A three-proton singlet for the ester methyl group appeared at δ_H 3.77, while methine signals at δ_H 3.94 and δ_C 62.1 could be assigned to C-21′. The partial structures of Unit A and Unit B were further confirmed by ^1H–^1H COSY and HMBC, as shown in Figure 1.

* Corresponding author. Tel./fax: +81 43 290 2901.
E-mail address: takayama@p.chiba-u.ac.jp (H. Takayama).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.07.145

Table 1
^{1}H and ^{13}C NMR data for **1** (in $CDCl_3$)

Position	δ_H (600 MHz)	δ_C (150 MHz)
NH	9.00 (1H, br s)	/
2	/	133.1
3	4.40 (1H, d, 11.8)	47.7
5	3.55 (2H, overlapped)	51.0
6	2.91 (1H, overlapped)	22.2
	2.78 (1H, d, 11.2)	
7	/	108.4
8	/	126.9
9	7.47 (1H, d, 7.9)	118.0
10	7.11 (1H, overlapped)	119.5
11	7.13 (1H, overlapped)	121.8
12	7.34 (1H, d, 7.9)	111.1
13	/	136.5
14	2.12 (1H, d, 12.6)	31.9
	1.54 (1H, overlapped)	
15	3.35 (1H, d, 4.4)	31.1
16	/	94.6
17	7.60 (1H, s)	146.5
18	0.62 (3H, d, 6.6)	19.2
19	2.24 (1H, m)	24.8
20	/	120.6
21	5.89 (1H, s)	139.6
CO_2Me	3.62 (3H, s)	50.7
CO_2Me	/	168.7
NH	8.85 (1H, br s)	/
2′	/	168.8
3′	2.94 (1H, overlapped)	45.4
	2.48 (1H, ddd, 12.4, 12.4, 4.4)	
5′	3.09 (1H, m)	53.6
	2.87 (1H, m)	
6′	1.89 (1H, dd, 13.2, 6.6)	44.2
	2.94 (1H, overlapped)	
7′	/	54.6
8′	/	135.2
9′	7.12 (1H, overlapped)	120.0
10′	6.89 (1H, dd, 8.0, 8.0)	121.2
11′	7.13 (1H, overlapped)	127.8
12′	6.79 (1H. d, 8.0)	110.2
13′	/	144.1
14′	1.78 (1H, br d, 13.5)	27.8
	1.58 (1H, overlapped)	
15′	3.39 (1H, s)	27.9
16′	/	96.2
18′	1.54 (2H, overlapped)	32.7
19′	3.19 (1H, ddd, 10.2, 7.4, 3.0)	68.0
20′	2.31 (1H, d, 10.2)	42.9
21′	3.94 (1H, br s)	62.1
CO_2Me'	3.77 (3H, s)	51.4
CO_2Me'	/	168.4

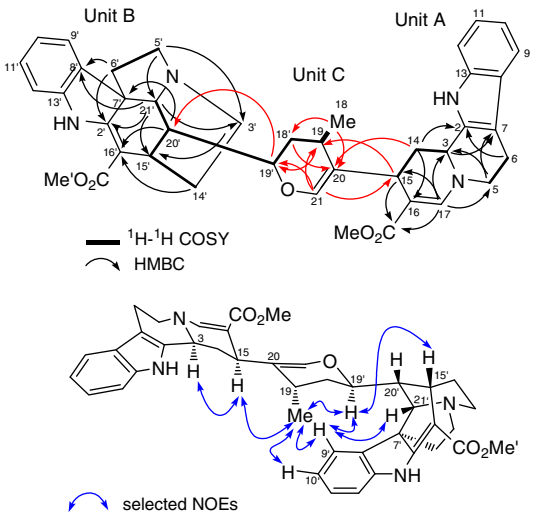


Figure 1. Selected HMBCs, COSYs, and NOEs of **1**.

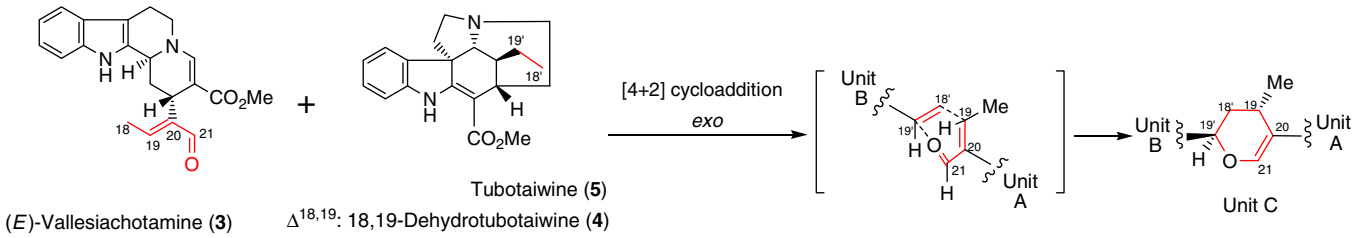
In addition to Units A and B, a methylene group (δ_H 1.54, δ_C 32.7), an oxygenated methine group (δ_H 3.19, δ_C 68.0), and two olefinic carbons (δ_C 120.6, δ_C 139.6) as revealed by ^{1}H NMR and ^{13}C NMR spectra comprised Unit C (dihydropyran ring). This is also well supported by HMBC ^3J-long range correlations of H-21/C-19′, H-21/C-19, H-18′/C-20, and H-19/C-19′. The remaining methyl protons (δ_H 0.62) that appeared as a doublet in the ^{1}H NMR spectrum were associated to C-19.

Thus, the three units depicted above were assembled according to HMBC and COSY correlations. C-20 of Unit C is linked to C-15 of monomeric Unit A, as identified by HMBC correlations from H-14 to C-20, H-15 to C-19, and H-21 to C-15, as well as NOE between Me-19 and H-15. The COSY spectrum suggested a direct linkage from Unit B to Unit C via C-20′ and C-19′. This was supported by the HMBC correlation of H-19′ to C-20′.

After assigning the constitution of the whole planar structure of new dimer **1**, we turned our attention to its stereochemistry. The relative configurations of the eight stereogenic centers were elucidated on the basis of observed NOE, as shown in Figure 1. The NOE observed between Me-19 and H-19′ in Unit C indicated that they were in axial orientation and cis relationship. To the best of our knowledge, except for Kopsiyunnanine A (**1**), the dimeric indole alkaloids family possessing a dihydropyran moiety has not been reported to date. We proposed a biosynthetic hypothesis for its formation from two monomeric indole alkaloids by the hetero Diels–Alder reaction (Scheme 1). Considering **1** as an *exo* product of this cycloaddition, we speculated that (*E*)-vallesiachotamine (**3**) and 18,19-dehydrotubotaiwine (**4**) [may be produced by the oxidation of tubotaiwine (**5**)] are the biogenetic precursors of the new dimer. The CD spectrum of **1** (Fig. 2) showed a curve that matched the sum of the CD spectra of (*E*)-vallesiachotamine (**3**) and tubotaiwine (**5**), implying that Unit A (3*S*,15*S*) and Unit B (7′*S*,15′*S*,21′*R*) share the same absolute configurations as their biogenetic precursors, respectively. In addition, the configurations of C-19, C-19′ in Unit C, and C-20′ in Unit B could be deduced from the NOE between Me-19 and H-9′ and Me-19 and H-10′, as well as between H-19′ and H-9′, and the large ^{1}H–^{1}H coupling constant (J = 10.2 Hz) between H-20′ and H-19′.

This is the first example of a dimeric indole alkaloid composed of vallesiachotamine (modified Corynanthe-type) and Aspidospermatan-type alkaloids. Kopsiyunnanine A (**1**) was evaluated for cytotoxic activity against two tumor cell lines. As a result, **1** showed a moderate cytotoxity to A-549 human lung adenocarcinoma cell line ($IC_{50} = 3.09 \times 10^{-3}$ μM) and HT-29 human colon adenocarcinoma grade II cell line ($IC_{50} = 2.05 \times 10^{-3}$ μM) compared to the positive control (docetaxel, $IC_{50} = 4.95 \times 10^{-7}$ μM to A-549 and $IC_{50} = 3.34 \times 10^{-7}$ μM to HT-29).

Kopsiyunnanine B (**2**)[10] was obtained as a light yellowish amorphous solid. It displayed a molecular ion peak at *m/z* 370.1879 [M^+] (calcd for 370.1892) in the HREIMS spectrum, which corresponded to the molecular formula $C_{21}H_{26}N_2O_4$ requiring 10 degrees of unsaturation. The UV spectrum showed absorption maxima at 284.5, 251.0, and 207.0 nm, suggesting a typical oxindole chromophore.[11] The ^{1}H NMR spectrum of **2** (Table 2) showed signals assignable to an indolic NH (δ_H 7.55, br s) and four aromatic protons (δ_H 7.40, d, H-9; δ_H 7.04, ddd, H-10; δ_H 7.20, ddd, H-11; δ_H 6.87, d, H-12), and the ^{13}C NMR spectrum showed a carbonyl carbon signal at δ_C 181.7. Together, these data indicate the presence of an unsubstituted A ring of the oxindole system. ^{1}H NMR and ^{13}C NMR spectra also confirmed the presence of a methyl ester group (δ_H 3.66, δ_C 51.7, δ_C 173.8), a methyl group (δ_H 0.75, δ_C 16.6), an aminomethine group (δ_H 2.97, δ_C 68.0), two aminomethylene groups (δ_H 3.23, δ_H 2.59, δ_C 54.0 and δ_H 2.99, δ_H 2.41, δ_C 47.4), an oxygenated methine group (δ_H 3.22, δ_C 69.0), and an oxygenated methylene group (δ_H 3.71, δ_H 3.58, δ_C 61.1).



Scheme 1. Hetero Diels–Alder cycloaddition of Unit C of **1** in hypothetical biogenesis.

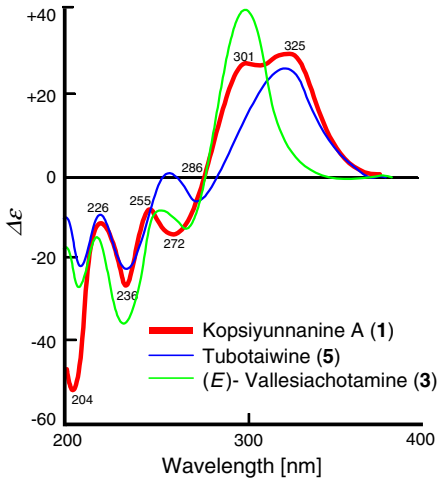


Figure 2. Circular dichroism (CD) spectra (MeOH, 18 °C) of **1**.

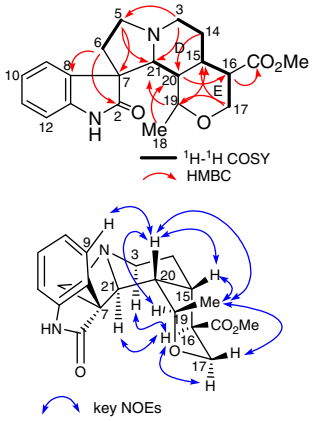


Figure 3. Selected HMBCs, COSYs, and NOEs of **2**.

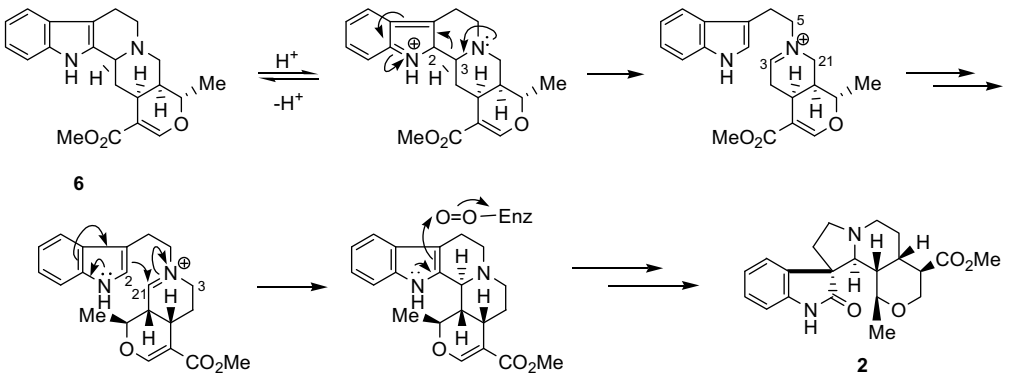
Table 2
^{1}H and ^{13}C NMR data for **2** (in $CDCl_3$)

Position	δ_H (500 MHz)	δ_C (125 MHz)
NH	7.55 (1H, br s)	/
2	/	181.7
3	2.99 (1H, m)	47.4
	2.41 (1H, m)	
5	3.23 (1H, m)	54.0
	2.59 (1H, ddd, 8.5, 8.5, 8.5)	
6	2.33 (1H, ddd, 13.0, 8.5, 2.0)	37.3
	2.00 (1H, ddd, 13.0, 9.0, 8.5)	
7	/	56.6
8	/	133.2
9	7.40 (1H, d, 7.5)	125.3
10	7.04 (1H, ddd, 7.5, 7.5, 1.0)	122.5
11	7.20 (1H, ddd, 7.5, 7.5, 1.0)	127.8
12	6.87 (1H, d, 7.5)	109.4
13	/	139.9
14	1.78 (1H, ddd, 14.0, 14.0, 4.5)	28.2
	1.57 (1H, dd, 14.0, 2.0)	
15	2.38 (1H, m)	29.2
16	3.06 (1H, ddd, 11.5, 11.5, 5.0)	40.9
17	3.71 (1H, dd, 11.5, 5.5)	61.1
	3.58 (1H, dd, 11.5, 11.5)	
18	0.75 (3H, d, 7.0)	16.6
19	3.22 (1H, m)	69.0
20	1.48 (1H, dd, 11.5, 4.5)	40.1
21	2.97 (1H, d, 11.0)	68.0
CO_2Me	/	173.8
CO_2Me	3.66 (3H, s)	51.7

^{1}H–^{1}H COSY analysis led to determination of the partial structures, which were then connected by HMBC correlations to give the gross structure of **2** (Fig. 3). HMBC correlations from H-5 to C-21 and C-7, and from H-6 to C-2 and C-8 allowed us to recognize the C(21)–N(4)–C(5)–C(6) moiety that is attached to the oxindole chromophore at C-7. The correlations from H-3 to C-21 and C-5 supported the direct linkage of the C(21)–N(4)–C(3)–C(14) moiety. Attachment of –$CHCH_3$ fragment to methine carbon C-20 was proven by the correlations from methyl protons H_3-18 to C-20 and from H-19 to C-21 and C-15. Furthermore, the correlations from H-17 to C-15 and C-19, and from H-20 to methine carbon (C-16) to which CO_2Me is attached established the partial formation of E ring that fused to D ring via C(20)–C(15) bridge. The remaining one degree of unsaturation required an oxygen to be embedded to complete E ring, according to the low-field chemical shifts of C-17 and C-19 at δ_C 61.1 and δ_C 69.0, respectively. Other correlations in the HMBC spectrum were in complete accordance with the proposed structure.

The relative configurations of compound **2** at different stereogenic centers were determined by analysis of ^{1}H–^{1}H coupling constants and NOE, as discussed below (Fig. 3). Because of the rigid structure, the configuration of C-20 as well as C-7 could be determined on the basis of the observed NOE between axial H-20 and aromatic H-9. The large coupling constant with H-20 and H-21 (J = 11.0 Hz) is characteristic of a trans–diaxial relationship. NOE of H-20/H-15 as well as Me-19/H-15 further suggested that D/E ring junction was cis, and both H-15 and Me-19 were axially oriented in E ring. The signal of H-16 appeared as ddd with two trans–diaxial ($J_1 = J_2$ = 11.5 Hz) disposition and a small coupling constant (J_3 = 5.0 Hz), depicting that H-16 was in axial relationship with H-15 and H-17β. This is also supported by the observed NOE of H-16/H-21, H-16/H-17α, and H-16/H-3α. The CD spectrum of **2** showed similar sign and magnitude of the Cotton effects to those of C/D trans Heteroyohimbine-type alkaloids with spiro-center C-7 (*S*) stereochemistry,[11] which gave a strong negative Cotton effect at around 280 nm. Therefore, according to the relative configuration established above, the absolute configurations at different chiral centers C-7, C-15, C-19, C-20, and C-21 of new alkaloid **2** were deduced to be all *S*, while that of C-16 was assigned *R*.

We proposed a possible biogenetic pathway to **2** (Scheme 2). Tetrahydroalstonine (**6**),[12] which was isolated from the same plant in our work, might be considered as the possible biogenetic origin.



Scheme 2. Possible biogenetic pathway of **2**.

Following cleavage of the C-2/C-3 bond of **6**, a rotation of the N-4/C-5 bond and subsequent isomerization of iminium function lead to a formation of a new bond between C-21 and C-2. After enzymatic oxidation, followed by reduction of double bond, the rearranged oxindole **2** would be afforded. The first example of an indole alkaloid with such a rotated ring D has been recently reported.[4e] Kopsiyunnanine B (**2**) appears to give the first instance of a Corynanthe-type oxindole alkaloid rearranged by a similar biogenetic pathway.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science, and Technology, Japan, Astellas Foundation for Research on Metabolic Disorders, and The Uehara Memorial Foundation.

References and notes

1. Middleton, D. J. *Harvard Pap. Bot.* **2000**, *9*, 89–142.
2. Feng, X. Z.; Kan, C.; Husson, H. P.; Potier, P. *J. Nat. Prod.* **1984**, *47*, 117–122.
3. Sevenet, T.; Allorge, L.; David, B.; Awang, K.; Hadi, A. H. A.; Kan-Fan, C.; Quirion, J. C.; Remy, F.; Schaller, H.; Teo, L. E. *J. Ethnopharmacol.* **1994**, *41*, 147–183.
4. For recent reports, see: (a) Sekiguchi, M.; Hirasawa, Y.; Zaima, K.; Hoe, T. C.; Chan, K. L.; Morita, H. *Heterocycles* **2008**, *76* and references cited therein; (b) Lim, K. H.; Kam, T. S. *Phytochemistry* **2008**, *69*, 558–561; (c) Subramaniam, G.; Hiraku, O.; Hayashi, M.; Koyano, T.; Komiyama, K.; Kam, T. S. *J. Nat. Prod.* **2008**, *71*, 53–57; (d) Subramaniam, G.; Choo, Y. M.; Hiraku, O.; Komiyama, K.; Kam, T. S. *Tetrahedron* **2008**, *64*, 1397–1408; (e) Lim, K. H.; Komiyama, K.; Kam, T. S. *Tetrahedron Lett.* **2007**, *48*, 1143–1145; (f) Lim, K. H.; Kam, T. S. *Helv. Chim. Acta* **2007**, *90*, 31–35; (g) Lim, K. H.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 8653–8655; (h) Lim, K. H.; Kam, T. S. *Org. Lett.* **2006**, *8*, 1733–1735; (i) Lim, K. H.; Low, Y. Y.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 5037–5039 and references cited therein.
5. For recent reports, see: (a) Kitajima, M. *J. Nat. Med.* **2007**, *61*, 14–23; (b) Kogure, N.; Ishii, N.; Kitajima, M.; Wongseripipatana, S.; Takayama, H. *Org. Lett.* **2006**, *8*, 3085–3088; (c) Kitajima, M.; Mori, I.; Arai, K.; Kogure, N.; Takayama, H. *Tetrahedron Lett.* **2006**, *47*, 3199–3202; (d) Takayama, H.; Kitajima, M.; Kogure, N. *Curr. Org. Chem.* **2005**, *9*, 1445–1464; (e) Takayama, H.; Mori, I.; Kitajima, M.; Aimi, N.; Lajis, N. H. *Org. Lett.* **2004**, *6*, 2945–2948.
6. Kopsiyunnanine A (**1**): UV (MeOH) λ_{max} nm ($\log\varepsilon$) 330.0 (3.99), 291.5 (4.56), 224.5 (4.55), 203.5 (4.49); FABMS (NBA) *m*/*z* 673 ($[M+H]^+$); HRFABMS (NBA/PEG) *m*/*z* 673.3359 ($[M+H]^+$, calcd for $C_{41}H_{44}N_4O_5$, 673.3390); $[\alpha]_D^{17}$ +258.8 (*c* 0.1, $CHCl_3$); CD (*c* 0.186 mmol/L, MeOH, 17 °C) $\Delta\varepsilon$ (λ nm) −51.85 (204), −24.30 (236), −14.24 (272), 0 (286), +22.34 (301), +25.65 (325), 0 (378).
7. Waterman, P. G.; Zhong, S. M. *Planta Medica* **1982**, *45*, 28–30.
8. Yamauchi, T.; Abe, F.; Padolina, W. G.; Dayrit, F. M. *Phytochemistry* **1990**, *29*, 3321–3325.
9. Rahman, A-u.; Zaman, K.; Rehman, H-u.; Malik, S. *J. Nat. Prod.* **1986**, *49*, 1138–1180.
10. Kopsiyunnanine B (**2**): UV (MeOH) λ_{max} nm ($\log\varepsilon$) 284.5 (3.29), 251.0 (3.61), 207.0 (4.19); EIMS *m*/*z* (%) 370 [M^+, 100]; HREIMS *m*/*z* 370.1879 ([M^+], calcd for $C_{21}H_{26}N_2O_4$, 370.1892); $[\alpha]_D^{18}$ −15.9 (*c* 0.1, $CHCl_3$); CD (*c* 0.74 mmol/L, MeOH, 18 °C) $\Delta\varepsilon$ (λ nm) −2.11 (216), 0 (225), +3.94 (237), 0 (249), −2.48 (261), −1.66 (285), 0 (305), +0.28 (324), 0 (360).
11. Kitajima, M.; Nakayama, T.; Kogure, K.; Wongseripipatana, S.; Takayama, H. *J. Nat. Med.* **2007**, *61*, 192–195.
12. Lounasmaa, M. *Tetrahedron* **1980**, *36*, 1607–1611.