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COMMUNICATION

Kopsihainanines A and B, two unusual alkaloids from *Kopsia hainanensis*†

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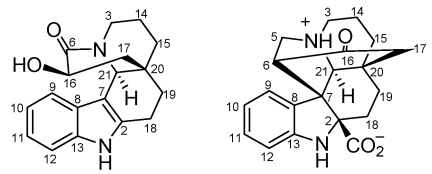
Kopsihainanine A (1), an unprecedented skeleton with a 6/5/6/6/6 pentacyclic rearranged ring system, together with a new biogenetically related compound, kopsihainanine B (2), were isolated from *Kopsia hainanensis*. Their structures were elucidated by means of spectroscopic methods. The absolute configuration of 1 was determined by ECD calculation.



The genus *Kopsia* (Apocynaceae) consists of 23 species of evergreen trees and shrubs that are widely distributed over Southeast Asia.[1] Four species are found in China.[2] Previous chemical investigations on this genus have led to the isolation of monomeric and dimeric monoterpenoid indoles as well as quinoline alkaloids which are considered to originate from the condensation of tryptophan with secologanin.[3] Many characteristic *Kopsia* alkaloids,[4,5] such as lapidilectine B,[6] mersicarpine,[7] kopsifoline,[8] and rhazinal,[9] have for a long time attracted great interest from synthetic organic chemists as challenging targets due to their marked diversity and complicated architectures. Pharmacological investigations on the crude and purified alkaloids from some *Kopsia* plants have demonstrated promising antitumor,[10] antimitotic,[11] antileishmanial,[12] and antitussive activities.[13] As part of searching for novel and bioactive indole alkaloids, we investigated the chemical constituents of *Kopsia hainanensis*, a native species used historically in folk medicine for the treatment of rheumatoid arthritis, pharyngitis, tonsillitis, and dropsy in Hainan Province, China.[14] A novel monoterpenoid indole alkaloid, kopsihainanine A (**1**), together with a new biogenetically related alkaloid, kopsihainanine B (**2**), have now been isolated from this plant. Among them, **1** possessed a rearranged methyl chanofruticosinate type alkaloid skeleton with a novel six-membered ring featuring C-6 connected to N-4 and showed inhibitory activity against AChE with IC_{50} values of 38.5 μM. In this paper, the isolation, structure elucidation, and biological activities of these new compounds are described.

The leaves and stems of *K. hainanensis* were collected in Hainan Province, P. R. China. A voucher species (No. 2009806) has been deposited in the State Key Laboratory of Applied Organic Chemistry (Lanzhou University, China). An air-dried and powdered sample (7 kg) was extracted with 95% MeOH (7 d × 3 times). The extract was partitioned between petroleum ether and 2% HCl solution. The acidic water-soluble materials, adjusted to pH 9–10 with 10% ammonia solution, were extracted with $CHCl_3$ to give an alkaloidal extract (25 g). The extract was subjected to silica gel column chromatography ($CHCl_3$: MeOH : Et_2NH, 1 : 0 : 0.002 to 0 : 1 : 0.002) to afford fractions A–H. Fraction C (10 g) was separated repeatedly by silica gel column chromatography ($CHCl_3$: MeOH : Et_2NH, 10 : 1 : 0.02) to yield **2** (24 mg). Fraction F (1.5 g) was separated repeatedly on RP-18 (MeOH : H_2O, 1 : 1) and Sephadex LH-20 (MeOH) columns to yield **1** (1.5 mg).

Kopsihainanine A (**1**),‡ isolated as white amorphous solid, possessed a molecular formula of $C_{18}H_{20}N_2O_2$ as established by HRESIMS ($[M + Na]^+$ at m/z 319.1410). The UV spectrum showed absorption maxima characteristic of an indole chromophore at 282 and 224 nm,[15] while the IR spectrum showed absorption bands due to NH (3395 cm^{-1}), OH (3308 cm^{-1}), and lactam (1658 cm^{-1}) functions.

The ^1H NMR spectrum revealed the existence of an ortho-disubstituted phenyl ring [δ_H 7.04 (1H, t, J = 8.4 Hz, H-10), 7.13 (1H, t, J = 8.4 Hz, H-11), 7.27 (1H, d, J = 8.4 Hz, H-12), 7.63 (1H, d, J = 8.4 Hz, H-9)] and an indolic NH group [δ_H 7.82 (1H, br s)] ($CDCl_3$). The ^{13}C NMR spectrum displayed a total of 18 carbon resonances which were assigned to six methylenes (δ_C 19.6, 21.8, 34.5, 37.4, 39.4, 53.8), six methines (δ_C 63.8, 68.7, 110.5, 119.8, 119.9, 122.0), and six quaternary carbons (δ_C 37.6, 109.9, 124.6, 133.0, 136.2, 185.8) ($CDCl_3$).

Partial structures **a** (C-9–C-12), **b** (C-3 and C-14–C-15), **c** (C-16–C-17), and **d** (C-18–C-19), shown in heavy lines in Fig. 1 were deduced from a detailed analysis of the ^1H–^1H COSY spectrum. The HMBC cross-peaks between H-9/C-7, H-10/C-8, H-18/C-7, and H-19/C-2 indicated that the C-8–C-7–C-2 unit connected the two units **a** and **d** together. The correlations from H-18,

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† Electronic supplementary information (ESI) available: Experimental details, NMR, MS, and IR spectra. Crystallographic data for the structure of **2**, CCDC reference number 797511. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob05724c

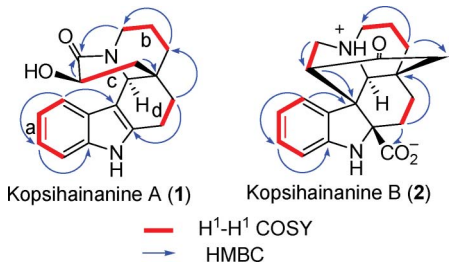


Fig. 1 Selected 2D NMR correlations for kopsihainanine A (**1**) and B (**2**).

H-19, H-17, H-16, H-14, and H-15 to C-20, suggested that the C-20 connected the three units **b**, **c**, and **d** together. The HMBC cross-peaks of H-21/C-7, H-21/C-8, and H-21/C-20 indicated that C-20 and C-7 were connected to C-21. The correlations from H-3 to C-21 and the chemical shifts of C-3 and C-21 implied that C-3 and C-21 were both connected to N-4. The HMBC cross-peaks of H-21/C-6 and H-17/C-6 established a six-membered ring of N(4)–C-6–C-16–C-17–C-20–C-21. The gross structure of **1** was thus elucidated to be as shown in Fig. 1, possessing a novel six-membered ring system including an α-hydroxyl lactam moiety.

The relative configuration of **1** was partially assigned by ROESY spectrum (Fig. 2). The ROESY correlations between H-21/H-19b and H-17b/H-19a indicated that C-21 and C-17 were on the opposite facial plane. The relative configuration of 16-OH was left unassigned by the available ROESY spectroscopic data. Given these conclusions, it was possible to prune a list of possible candidate stereostructures to just those assembled in Fig. 3, coded as **i**–**iv**.

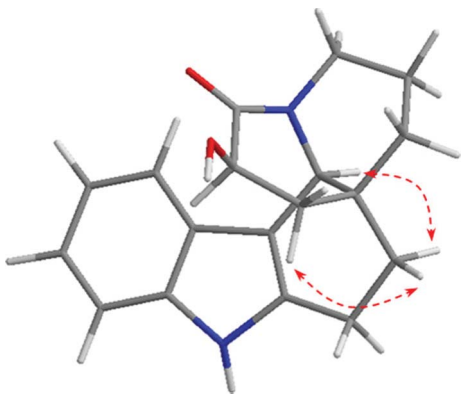


Fig. 2 Selected ROESY correlations (arrow) and relative stereochemistry for kopsihainanine A (**1**).

The absolute configuration of **1** was studied by comparing the actual electronic circular dichroism (ECD) trace of kopsihainanine A with those predicted for candidates **i**–**iv** using AM1[16] calculations and ZINDO[17] method. This approach is becoming a powerful tool in the absolute configuration analysis of natural products.[18] The CD calculations were performed by Gaussian 09 package. AM1 calculations have been performed to optimize ground/excited-state geometries. ZINDO method was used to provide further information on simulated UV-vis and CD spectra, the peak position and oscillator of simulated UV-vis and CD spectra were corrected by comparison with the maximum peak in experimental UV-vis and CD spectra. The CD spectrum of **1**

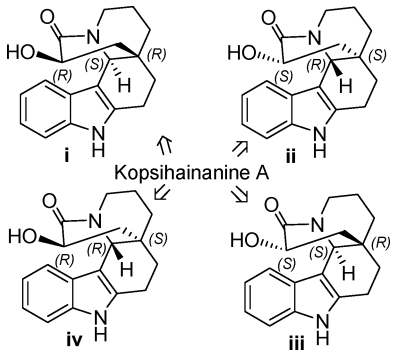


Fig. 3 Matrix of possible kopsihainanine A (**1**) configurations.

and that calculated for the molecule having 16*R*, 20*R*, 21*S* (**i**) were in good agreement (Fig. 4). Therefore, the absolute configuration of **1** was deduced to be 16*R*, 20*R*, 21*S*.

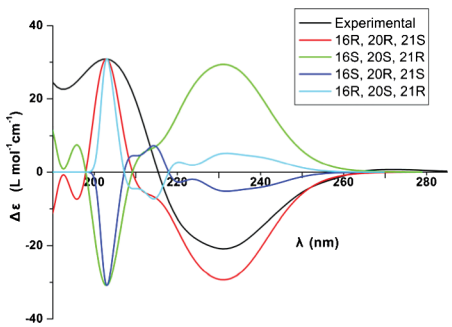


Fig. 4 Experimental CD spectrum of kopsihainanine A (**1**) overlaid with calculated spectra for structures shown in Fig. 3: (16*R*, 20*R*, 21*S*)-**i**, (16*S*, 20*S*, 21*R*)-**ii**, (16*S*, 20*R*, 21*S*)-**iii**, (16*R*, 20*S*, 21*R*)-**iv**.

Kopsihainanine B (**2**)§¶ was obtained as colorless crystals ($CHCl_3$). The UV spectrum showed absorption maxima at 210, 243, and 291 nm, characteristic of a dihydroindole chromophore, while the IR spectrum indicated the presence of NH (3384 and 3331 cm^{-1}), ketone (1714 cm^{-1}), and carboxylate (1578 cm^{-1}) functions. The molecular formula was established as $C_{20}H_{22}N_2O_3$ by HRESIMS ($[M + H]^+$ at m/z 339.1700). The NMR data of **2** was generally similar to that of methyl N1-decarbomethoxy chanofruticosinate,[19] except that a usual methyl ester group is now replaced by a carboxylate function (δ_C 178.3), as supported by the lack of OCH_3 group in the ^1H and ^{13}C NMR and the presence of a carboxylate function as indicated by the IR absorption band at 1578 cm^{-1}. A single-crystal X-ray diffraction confirmed the structure of **2** possessing a carboxylate group (Fig. 5).

A possible biogenetic pathway for the production of kopsihainanine A (**1**) from kopsihainanine B (**2**) was proposed. Due to the zwitterionic structure of the alkaloid **2**, as shown in Scheme 1, a strain-releasing decarboxylative fragmentation resulted in the formation of tricyclic enone intermediate **I**,[20] followed by the selective terminal olefin oxidation, giving the α-ketoaldehyde **II**. With *in situ* generation of the hemi-aminoacetal **III** *via* an intramolecular hemiacetalization, a subsequent keto-enol tautomerization delivered the bisenol intermediate **IV**, which can thermodynamically transform to α-hydroxy amide-containing kopsihainanine A (**1**) with an unprecedented 6/5/6/6/6 pentacyclic skeleton.

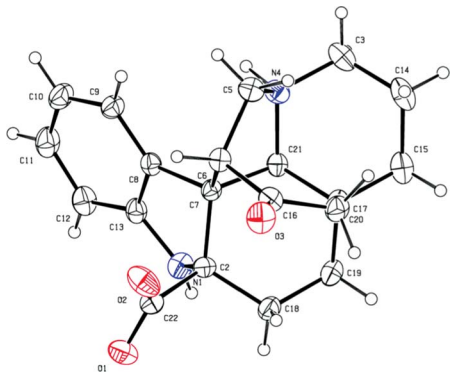
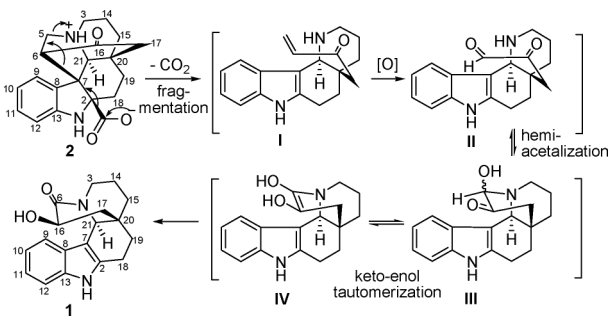


Fig. 5 X-ray structure of kopsihainanine B (**2**).



Scheme 1 Possible biogenetic route for Kopsihainanine A (**1**).

Compounds **1** and **2** were tested for AChE inhibiting activity by Ellman's method in 96-well microplates.[21] The results showed that **1** and **2** exhibited weak inhibitory activity with IC_{50} values of 38.5 and 50.6 μM. Unfortunately, intensive pharmacological investigation on **1** and **2** could not be carried out due to the limited amounts of these materials available.

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Notes and references

‡ Kopsihainanine A (**1**): white amorphous solid; $[\alpha]_D^{25}$ +60 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 282 (3.40), 224 (3.98) nm; IR (KBr) ν_{max} 3395, 3308, 2924, 1658, 1463, 1090, 737 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.26 (dd, 13.8, 10.8, H-17b), 1.60 (br dd, 14.4, 3.6, H-14b), 1.71 (m, H-15a, 15b), 1.82 (ddd, 17.4, 11.4, 6.0, H-19b), 1.92 (br dd, 17.4, 6.6, H-19a), 1.95 (ddd, 14.4, 6.0, 4.8, H-14a), 2.39 (dd, 13.8, 8.4, H-17a), 2.78 (br dd, 16.8, 6.6, H-18b), 3.09 (m, H-18a), 3.24 (td, 13.2, 3.6, H-3b), 3.58 (d, 6.0, 16-OH), 4.02 (ddd, 10.8, 8.4, 6.0, H-16), 4.36 (s, H-21), 4.43 (dd, 13.2, 6.0, H-3a), 7.04 (t, 8.4, H-10), 7.13 (t, 8.4, H-11), 7.27 (d, 8.4, H-12), 7.63 (d, 8.4, H-9), 7.82(br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ (19.6, C-18), (21.8, C-14), (34.5, C-19), (37.4, C-17), (37.6, C-20), (39.4, C-15), (53.8, C-3), (63.8, C-21), (68.7, C-16), (109.9, C-7), (110.5, C-12), (119.8, C-10), (119.9, C-9), (122.0, C-11), (124.6, C-8), (133.0, C-2), (136.2, C-13), (185.8, C-6); HRESIMS *m/z* 319.1410 $[(M+Na)^+$, calcd for $C_{18}H_{20}N_2O_2Na$, 319.1417).

§ Kopsihainanine B (**2**): colorless crystals; mp 213 °C; $[\alpha]_D^{25}$ +36 (*c* 0.50, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 291 (3.15), 243 (3.47), 210 (2.76) nm; IR (KBr) ν_{max} 3384, 3331, 2924, 1714, 1606, 1578, 1456, 1365, 1057, 754 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.23 (br d, 7.8, H-19b), 1.34 (overlapped, H-14b, H-15b), 1.48 (br d, 13.8, H-15a), 1.70 (overlapped, H-18b, H-19a), 1.88 (overlapped, H-14a, H-18a), 1.97 (d, 18.6, H-17b), 2.51 (s, H-21), 2.73 (d, 18.6, H-17a), 2.84 (d, 10.8, H-5b), 2.95 (m, H-3a, 3b), 3.33(d, 6.6, H-6), 3.78 (dd, 10.8, 6.6, H-5a), 6.64 (t, 7.8, H-10), 6.68 (d, 7.8, H-12), 6.99 (t, 7.8, H-11), 7.03 (d, 7.8, H-9); ^{13}C NMR (125 MHz, $CDCl_3$): δ (17.3, C-14), (27.8, C-18), (34.6, C-15), (35.3, C-19), (36.0, C-20), (42.7, C-17), (46.7, C-3), (52.7, C-5), (55.3, C-6), (57.7, C-7), (68.3, C-21), (74.0, C-2), (110.3, C-12), (119.1, C-10), (123.6, C-9), (127.7, C-11), (133.7, C-8), (148.7, C-13), (178.3, CO_2^-), (210.1, C-16); HRESIMS *m/z* 339.1700 $([M+H]^+$, calcd for $C_{20}H_{23}N_2O_3$, 339.1703).

¶ Crystal data for kopsihainanine B (**2**): $C_{20}H_{22}N_2O_3{\cdot}3H_2O$, M = 392.44, orthorhombic, space group $P2_12_12_1$; a = 11.3829(13) Å, b = 12.3833(15) Å, c = 13.4311(16) Å, $\alpha = \beta = \gamma = 90.00°$, V = 1893.2(4) $Å^3$, Z = 4, D_{calc} = 1.377 g cm^{-3}, λ = 0.71073 Å, μ (Mo Kα) = 0.076 mm^{-1}, $F(000)$ = 840, and T = 296(2) K, crystal dimensions 0.35 × 0.32 × 0.30 mm was selected for X-ray analysis. The SMART program was used to make data corrections. A total of 11744 reflections, collected in the range $2.24° \leq \theta \leq 25.50°$, yielded 3496 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on F^2 values for $2421I > 2\sigma(I)$. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0786, R_w = 0.0898 with goodness-of-fit = 1.101. Scattering factors were taken from International Tables for X-ray Crystallography. Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 797511). Copies of these data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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