Note

Two New Alkaloids from the Roots of *Baphicacanthus cusia*

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Phytochemical investigation of the root of *Baphicacanthus cusia* (Nees) Bremek afforded two new alkaloids, baphicacanthin A (1) and baphicacanthin B (2), along with 28 known compounds. The chemical structures of these compounds were elucidated on the basis of one and two dimensional (1D/2D)-NMR and high resolution (HR)-MS spectral evidence.

Key words *Baphicacanthus cusia*; baphicacanthin A; baphicacanthin B; alkaloid; structural elucidation

Baphicacanthus cusia (Nees) Bremek which is distributed in Southern China is the only plant of *Baphicacanthus* (Acanthaceae). Its root is being used as a traditional Chinese medicine named 'Nan-Ban-Lan-Gen (NBLG).'[1] The nature and taste of this medicinal material is cold and bitter, and the action is to clear away heat and toxicity in human body. As a frequently used Chinese herbal medicine for anti-viral treatment, it was recorded in "People's Republic of China Pharmacopoeia (2015)."[2] It has been also listed as one of the 8 major anti-severe acute respiratory syndrome (SARS) medicines during the outbreak of SARS in 2003.[3]

Till now indigoid indole alkaloids, quinazolinone alkaloids, monoterpenes, triterpenes, flavonoids, sterols, anthraquinones, benzoxazinones and lignans have been reported from *Baphicacanthus cusia*.[4] Since diversified components in Chinese herbal medicines often act *via* multiple modes to create a synergistic effect,[5] this paper deals with the elucidation of various chemical constituents in NBLG based on systematic isolation and purification, in order to provide chemical basis for the multiple-targeting effect of NBLG.

Results and Discussion

Isolation and purification of the extract of *B. cusia* afforded 30 compounds, including two new compounds (**1**, **2**). On the basis of the comparison of their NMR spectroscopic data with those reported in the literature, the 28 known compounds were identified to be 2-benzoxazolinone (**3**),[6] tryptanthrin (**4**),[7] 2-hydroxy-1,4-benzoxazin-3-one (**5**),[8] 3-(2′-hydroxyphenyl)-4(3*H*)-quinazolinone (**6**),[9] 1*H*-indole-3-carbaldehyde (**7**),[10] benzouracil (**8**),[11] 2*H*-1,4-benzoxazin-3-one (**9**),[12] 3-carboxyindole (**10**),[13] acanthaminoside (**11**),[14] 4(3*H*)-quinazolinone (**12**),[15] deoxyvasicinone (**13**),[16] acanthaminoside isomer (**14**),[14] lupeol (**15**),[17] betulin (**16**),[18] betulinic acid (**17**),[19] ursolic acid (**18**),[20] lup-20(29)-en-3*β*,30-diol (**19**),[21] maslinic acid (**20**),[22] guaiacylglycerol-*β*-ferulic acid ether (**21**),[23] (2*S*,3*R*,4*S*)-lyoniresinol-3*α*-*O*-*β*-D-glucopyranoside (**22**),[24] (2*R*,3*S*,4*R*)-lyoniresinol-3*α*-*O*-*β*-D-glucopyranoside (**23**),[24] (+)-5,5′-dimethoxy-9-*O*-*β*-D-glucopyranosyl secoisolariciresinol (**24**),[25] tyrosol (**25**),[26] *β*-hydroxy-benzenepentanoic acid (**26**),[27] acteoside (**27**),[28] acteoside isomer (**28**),[28] loliolide (**29**),[29] hispiduloside (**30**).[30] Among these, compounds **3**–**14** belong to alkaloids (Fig. 1), **15**–**20** belong to triterpenoids, **21**–**24** belong to lignans, **25**–**28** belong to phenylethanoids, **29** is a sesquiterpene lactone and **30** is a flavonoid. Compounds **5**–**9**, **11**, **14**, **19**, **20**, **21**, **25** and **26** were isolated from this plant for the first time.

Compound **1** was obtained as brown yellow amorphous powder. Its high resolution-electrospray ionization (HR-ESI)-MS showed a positive pseudo-molecular ion peak at *m*/*z* 258.0769 ($[M+H]^+$; Calcd for 258.0761), corresponding to $C_{14}H_{12}NO_4$ having 10 degrees of unsaturation. The ^{1}H-NMR spectrum suggested the presence of one 1,2-disubstituted benzene ring (δ_H 7.82 (dd, J=1.5, 8.0 Hz), 7.62 (dt, J=1.6, 8.0 Hz), 7.47 (dt, J=1.4, 8.0 Hz), 7.54 (dd, J=1.2, 8.0 Hz)), an olefinic proton (δ_H 6.76 (s)) and two methoxyl groups (δ_H 4.00 (s), 4.01 (s)). From the above evidence, **1** was supposed to be analogous to questiomycin A[31] (Chart 1). The ^{13}C-NMR spectrum of **1** showed total 14 carbons signals. While ^{13}C-NMR signals of the A-ring of **1** were good in accordance with those of questiomycin A,[31] a significant change was observed on the C-ring. Heteronuclear multiple bond connectivity (HMBC) correlations of H-1′ (δ_H 4.00 (s)) to C-2 (δ_C 156.8) and H-2′ (δ_H 4.01 (s)) to C-4 (δ_C 136.1) indicated the methoxyl group 1′-OMe was located at C-2 and 2′-OMe was located at C-4, which was also supported by the HMBC correlation of H-1′ (δ_H 4.00 (s)) to C-1 (δ_C 104.2) (Fig. 2). Consequently, compound **1** was determined as 2,4-dimethoxyl-3*H*-phenoxazin-3-one and named as baphicacanthin A.

Compound **2** was obtained as yellow amorphous powder, $[\alpha]_D^{25}$ +55.7 (c=0.620, MeOH). Its HR-ESI-MS displayed a pseudo-molecular ion peak at *m*/*z* 457.1233 ($[M-H]^-$; Calcd for 457.1253), corresponding to $C_{22}H_{21}N_2O_9$ having 9 degrees of unsaturation. The anomeric proton at δ_H 5.27 (d, J=7.7 Hz, 1H), with the carbon signals at δ_C 62.7, 71.3, 73.6, 77.8, 78.3 and 105.4 were indicative of a *β*-glucopyranosyl moiety. The acid hydrolysis of compound **2** in 1 N HCl produced glucose whose absolute configuration was determined as D form based on the comparison with D- and L-glucose standards on LC-MS.[33] The ^{1}H- and ^{13}C-NMR resonances corresponding to the aglycone moiety consisted of substituted indole (δ_H 12.98

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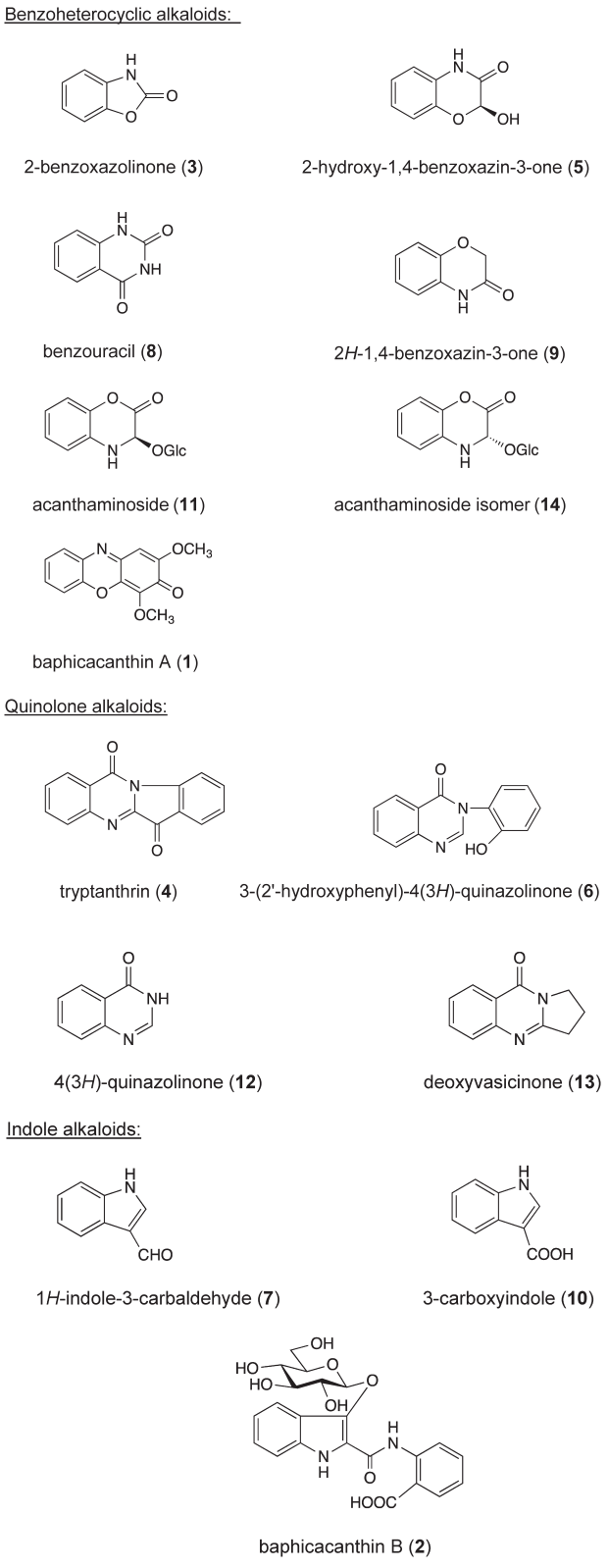

Fig. 1. Structures of Isolated Alkaloids from *B. cusia*

(s), 7.90 (d, J=8.0 Hz), 7.09 (t, J=8.0 Hz), 7.27 (t, J=8.0 Hz), 7.43 (d, J=8.0 Hz) and δ_C 120.2, 138.8, 120.9, 119.9, 121.6, 126.1, 113.5, 135.8) and *o*-carboxamidebenzoate (δ_H 12.75 (s), 8.09 (d, J=8.0 Hz), 7.58 (t, J=8.0 Hz), 7.19 (t, J=8.0 Hz), 8.71 (d, J=8.0 Hz) and δ_C 161.5, 141.8, 119.9, 132.3, 123.9, 134.4, 122.9, 171.2). The HMBC correlation observed between H-1

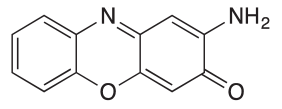

Chart 1

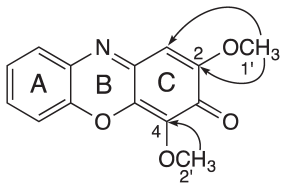

Fig. 2. Selected HMBC Correlations of Baphicacanthin A (**1**)

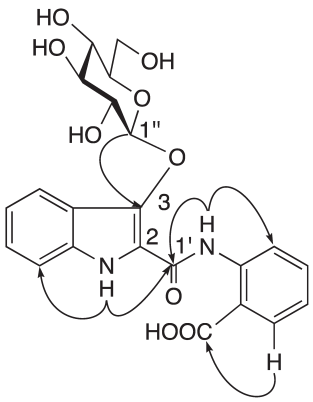

Fig. 3. Selected HMBC Correlations of Baphicacanthin B (**2**)

(δ_H 12.98 (s)) and C-1′ (δ_C 161.5) suggested that the *o*-carboxamidobenzoate moiety linked with C-2 of indole. The position of the glucosyl unit at C-3 was supported by the HMBC correlation between H-1″ (δ_H 5.27 (d, J=7.7 Hz)) and C-3 (δ_C 138.8) (Fig. 3). Therefore, compound **2** was elucidated as 2-[[[(3-β-D-glucopyranosyloxy)-1*H*-indol-2-yl]carbonyl]amino]benzoic acid and named as baphicacanthin B.[32] This compound is a demethyl (carboxylic acid) analogue of cephalandole C.

Conclusion

Systematic isolation and purification of chemical constituents of NBLG led to the isolation of 30 constituents, among which 2 alkaloids are new compounds which have not been previously reported, and 12 compounds were isolated from NBLG for the first time. This chemical study revealed notable structure diversity of the chemical constituents in NBLG which might be the chemical basis for the well-recognized anti-virus effect of NBLG.

Experimental

General Experimental Procedures TLC: Kieselgel 60 F_{254} plates (0.2 mm thick, Merck KGaA Corporation, Germany); visualized by UV light (254, 366 nm) and by spraying with 10% H_2SO_4 reagent. Column chromatography (CC): silica gel 60 (200–300 mesh, Merck KGaA Corporation) and Reverse Phase-18 (RP-18) (45 μm, Merck KGaA Corporation). Medium Pressure Preparative Liquid Chromatography:

BUCHI MPLC System using a RP-18 column (SilicBond C18, 36×460 mm i.d., 40–63 μm particle size (Silicycle)). Preparative and semi-preparative HPLC: Lab Alliance system with a YMC-Pack ODS-A column (10 μm, 250×10 mm) and a Vision HT C18 polar column (5 μm, 22×250 mm, Grace, U.S.A.). Optical rotations: Rudolph Research Analytical Autopol I automatic polarimeter (Na 589 nm); in MeOH. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) spectra: A Bruker Ascend 600 NMR spectrometer; in CD_3OD and pyridine-d_5; at ambient temperature; coupling constants J in Hz, and chemical shifts in δ [ppm]. HR-ESI-MS: Agilent 6230 accurate mass time-of-flight (TOF) mass spectrometer (U.S.A.) equipped with an ESI, coupled to an UHPLC systerm performed on an Agilent 1290 system using an Eclipse XDB-C18 column (3.0×150 mm, Agilent); in m/z.

Plant Material The root of *Baphicacanthus cusia* (Nees) Bremek was collected from Honghe, Yunnan, China in October 2012. The plant was authenticated by Dr. Zhifeng Zhang (Macau University of Science and Technology).

Extraction and Isolation Air-dried roots of *Baphicacanthus cusia* (Nees) Bremek (3 kg) were cut into small pieces and refluxed with 80% aqueous EtOH (30, 24, 18 L). The extract was suspended in water, and partitioned with ethyl acetate (1 L each) and *n*-BuOH (1 L each) successively to yield the ethyl acetate layer (44 g), *n*-BuOH layer (48 g) and H_2O layer (62 g).

The ethyl acetate layer was subjected to silica gel CC (35×5 cm) using a gradient mixture of ethyl acetate–petroleum ether (9 : 1 to 5 : 5) as eluent to afford Frs. 1–13. Fraction 3 (6.84 g) was purified by CC over silica gel (*n*-hexane–ethyl acetate=9 : 1 to 5 : 5) to yield compound **15** (2.3 g). Fraction 4 (3.0 g) was subjected to chromatography on RP-18 CC (MeOH–H_2O=9 : 1 to 7 : 3) and silica gel CC (*n*-hexane–ethyl acetate=9 : 1 to 6 : 4) to yield compounds **3** (2.3 mg), **4** (3.2 mg) and **16** (4.0 mg). The combined Fr. 5 (1.2 g) and Fr. 6 (0.6 g) were separated by RP-18 CC (MeOH–H_2O=9 : 1 to 6 : 4) to yield compounds **17** (2.0 mg), **18** (3.0 mg), and **19** (3.0 mg). Fraction 7 (2.0 g) was separated by RP-18 CC with MeOH–H_2O (0 : 100 to 100 : 0) and MeOH–CH_3COCH_3 (100 : 0 to 50 : 50) to give 12 sub Frs. 7-1–7-12. Sub Fr. 7-4 (71.0 mg) afforded compounds **25** (1.0 mg) and **5** (2.0 mg), sub Fr. 7-6 (54.0 mg) afforded compounds **6** (1.5 mg), **29** (1.0 mg) and **7** (1.5 mg) and sub Fr. 7-8 (63.0 mg) afforded compounds **1** (1.5 mg) and **20** (2.0 mg), all by semi-preparation HPLC (MeCN–H_2O). Fraction 8 (1.4 g) was separated by MPLC (eluents A: H_2O, B: CH_3OH, gradient: B 20% at 0, B 60% at 45 min, B 80% at 65 min) and semi-preparative HPLC eluted with MeCN–H_2O (30 : 70) to obtain compounds **8** (1.5 mg), **9** (1.0 mg) and **10** (1.0 mg). Fraction 11 (1.7 g) was separated by MPLC (eluents A: H_2O, B: CH_3OH, gradient: B 20% at 0, B 60% at 45 min, B 80% at 65 min) and semi-preparative HPLC eluted with MeCN–H_2O (30 : 70) to obtain compounds **11** (2.1 mg), **27** (5.0 mg), **28** (1.0 mg), **12** (2.0 mg), and **13** (1.5 mg). Fraction 12 (0.9 g) was separated by MPLC (eluents A: H_2O, B: CH_3OH, gradient: B 20% at 0, B 60% at 45 min, B 80% at 65 min) to afford compound **30** (5.1 mg).

The *n*-BuOH layer (48 g) was chromatographed on MCI CHP20P (55×3.5 cm; analytical TLC control) eluting with 2 L mixtures of MeOH–H_2O (10 : 90 to 100 : 0) to give Frs. 1–10. Fraction 8 (1.4 g) was separated by MPLC (eluents A: H_2O, B: CH_3OH, gradient: B 20% at 0, B 60% at 45 min, B 80% at 65 min) and preparative HPLC eluted with MeCN–H_2O (30 : 70) to obtain compounds **14** (1.0 mg), **21** (1.0 mg), **2** (2.0 mg) and **26** (1.0 mg). Fraction 10 (1.5 g) was separated by MPLC (eluents A: H_2O, B: CH_3OH, gradient: B 20% at 0, B

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** in MeOD and **2** in Pyridine-d_5

Compound **1**			Compound **2**		
Position	δ_C	δ_H Mult. (J in Hz)	Position	δ_C	δ_H Mult. (J in Hz)
1	104.2	6.76 s	1		12.98 s
2	156.8		2	120.2	
3	175.9		3	138.8	
4	136.1		4	120.9	
4a	138.0		5	119.9	7.90 d (8.0)
5a	143.3		6	121.6	7.09 t (8.0)
6	129.4	7.82 dd (1.5, 8.0)	7	126.1	7.27 t (8.0)
7	131.2	7.62 dt (1.6, 8.0)	8	113.5	7.43 t (8.0)
8	125.4	7.47 dt (1.4, 8.0)	9	135.8	
9	116.3	7.54 dd (1.2, 8.0)	1′	161.5	
9a	133.5		2′	141.8	12.75 s
10a	148.4		3′	119.9	
1′	56.5	4.00 s 3H	4′	132.3	8.09 d (8.0)
2′	60.9	4.01 s 3H	5′	123.9	7.58 d (8.0)
			6′	134.4	7.19 t (8.0)
			7′	122.9	8.71 d (8.0)
			8′	171.2	
			1″	105.4	5.27 d (7.7)
			2″	74.6	3.89 m
			3″	77.8	3.51 t (8.7)
			4″	71.3	3.39 m
			5″	78.3	3.42 t (8.7)
			6″	62.7	α 3.61 d (5.0)
					β 3.80 dd (11.7, 2.3)

60% at 45 min, B 80% at 65 min) and preparative HPLC eluted with MeCN–H_2O (30 : 70) to yield compounds **22** (0.5 mg), **23** (1.0 mg) and **24** (1.5 mg).

Baphicacanthin A (=2,4-dimethoxyl-3*H*-phenoxazin-3-one; **1**). UV λ_{max} (MeOH) nm (log ε): 223 (2.8), 255 (2.5), 385 (2.7); IR (KBr) cm^{-1}: 3069, 1638, 1588; ^1H- and ^{13}C-NMR see Table 1. HR-ESI-MS: 258.0769 ($[M+H]^+$, $C_{14}H_{13}NO_4^+$; Calcd for 258.0761).

Baphicacanthin B (=2-[[[(3-β-D-glucopyranosyloxy)-1*H*-indol-2-yl]carbonyl]amino]-benzoic acid; **2**). White amorphous powder. $[\alpha]_D^{25}$ +55.7 (c=0.620, MeOH); UV λ_{max} nm (MeOH) (log ε): 207 (2.1), 227 (2.0), 259 (1.7), 300 (1.8), 316 (1.8); IR (KBr) cm^{-1}: 3399, 1684, 1647, 1586; ^1H- and ^{13}C-NMR see the Table 1. HR-ESI-MS: 457.1293 ($[M-H]^-$, $C_{22}H_{20}N_2O_9^-$; Calcd for 457.1253).

Determination of the Absolute Configuration of Sugar The absolute configuration of glucose in compound **2** was determined according to the reported protocol.[33] Briefly, compound **2** (0.45 mg) was dissolved in MeOH (0.1 mL) and then added 1 N HCl (0.1 mL). The solution was heated at 80°C for 4 h and then the solvent was removed under N_2. The residue was dissolved in pyridine (0.1 mL) along with L-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60°C for 1 h. A 20 μL solution of phenyl isothiocyanate was added to the mixture and heated at 60°C for another 1 h. Twenty microliter of the reaction mixture was diluted to 500 μL in MeOH and then analyzed by using UHPLC-TOF MS (Eclipse XDB-C18 column (3.0×150 mm, Agilent), ACN–H_2O=20 : 80, flow rate at 0.35 mL/min). D- and L-Glucose standards were treated in the same way. The retention time of glucose derivative obtained from acid hydrolysates of compound **2** was 21.65 min. (The retention times of derivatives of D- and L-glucose standards were 21.77, 20.38 min, respectively.)

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Conflict of Interest The authors declare no conflict of interest.

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