Isolation and Characterization of Brachystemidines A−E, Novel Alkaloids from *Brachystemma calycinum*

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Five novel alkaloids, brachystemidines A−E (**1**−**5**), were isolated from the roots of *Brachystemma calycinum*. Their structures were established by spectral data, especially by 1D and 2D NMR techniques. The crystal structure of brachystemidine D was determined via X-ray diffraction analysis.

Brachystemma calycinum D. Don (Caryophyllaceae) is the only member of the genus *Brachystemma*. It is sporadically distributed in the southwest of China.[1] In China it has been used as a folk medicine for rheumatism, limb numbness, impotence, and edema of the feet.[2] Our chemical investigation on *B. calycinum* has led to the isolation of five novel alkaloids (**1**−**5**) named brachystemidines A−E.

Brachystemidine A (**1**) was obtained as a white solid from the EtOAc extract of the roots of *B. calycinum*. The molecular formula, $C_{15}H_{18}N_2O_5$, which indicated eight unsaturations, was deduced from HREIMS at *m*/*z* 306.1238 (calcd. 306.1218) and from the ^{13}C NMR and DEPT spectra. The ^1H and ^{13}C NMR data (Table 1), including $^1H-^1H$ COSY, HMQC, and HMBC (Table 1) spectra, suggested that **1** was an alkaloid consisting of three ring systems, a pyrrole, a dihydrofuran, and a pyrrolidone residue. The 2-substituted pyrrole fragment was deduced from comparison of ^{13}C NMR spectral data with 3-furfuryl pyrrole-2-carboxylate.[3] The $^1H-^1H$ cross-peaks between H_a-3″ (*δ* 2.17, m) and H_b-4″ (*δ* 1.93, m), between H_b-3″ (*δ* 2.43, m) and H_a-4″ (*δ* 1.97, m), and between H_a-4″ (*δ* 1.97, m) and H-5″ (*δ* 4.74, m), together with H-3″, H_b-4″, and H-5″, all correlating with the amide carbonyl group (*δ* 175.1) in the HMBC spectrum, indicated the presence of a substituted pyrrolidone. Protons (*δ* 3.11, 3H, s) correlating with C-5″ (*δ* 87.7) in the HMBC spectrum implied that one OMe was linked with C-5″. The presence of a substituted 2,5-dihydrofuran moiety was indicated in the HMBC spectrum by correlations of H_b-5′ (*δ* 4.73) with C-2′ (*δ* 86.5), C-3′ (*δ* 127.4), and C-4′ (*δ* 134.3). The signals resonating at *δ* 86.5 and 74.5 were indicative of oxygen-bearing atoms. That one CH_2 bearing an oxygen atom (H-6′) was linked to the 4′-position of the dihydrofuran ring and was supported by its HMBC correlations with C-3′ (*δ* 127.4) and C-4′ (*δ* 134.3), in addition to C-6 (*δ* 160.0). The linkage of the pyrrolidone and dihydrofuran residue was accomplished by observations of HMBC correlations between H-2′ (*δ* 6.38) and C-5″ (*δ* 87.7) and between H-5″ (*δ* 4.74) and C-2′ (*δ* 86.5). A carbonyl ester bond was reasonable for connecting the dihydrofuran and pyrrole. The obvious $^1H-^1H$ cross-peaks of H_b-5′ (*δ* 4.73) and H-2′ (*δ* 6.38) indicated zigzag coupling. Such a phenomenon required the two protons to be positioned in one plane, and hence, the five-membered ring should adopt an envelope conformation. Thus, the structure of brachystemidine A was assigned as **1**.

The EIMS and ^1H and ^{13}C NMR spectra (Tables 2 and 3) of **2** were surprisingly similar to those of **1**, although they were recorded in different solvents. However, their TLC behavior was different in three different solvent systems, implying that **2** was isomeric with **1**. The clear HMBC correlations of H_a-6′ (*δ* 4.73) and H_b-6′ (*δ* 4.71) both with C-2′ (*δ* 87.5) in **2** and their absence in **1** suggested that **2** was the 3′-positional isomer of **1**. Likewise, the conformation of the substituted 2′,5′-dihydrofuran was assumed via a zigzag coupling of H_b-5′ (*δ* 4.66) and H-2′ (*δ* 6.60). Hence, the structure of brachystemidine B was assigned as **2**.

The similarities of the ^1H and ^{13}C NMR data (Tables 2 and 3) of **3** with **1** and **2** suggested that **3** was a derivative of **1** or **2**. The EIMS of **3** displayed 16 amu more than **1** or **2**, besides a significant downfield shift of C-3″ which appeared at *δ* 68.6 in **3** rather than at *δ* 29.3 as in **2** or at *δ* 28.8 as in **1**; this suggested a hydroxyl group was located at C-3″ in **3**. The HMBC correlations of H-6′ (*δ* 4.76 and 4.97) with C-2′ (*δ* 88.3) indicated that the 3′-position of **3** was substituted. The conformation of the 2′,5′-dihydrofuran was established through the $^1H-^1H$ interactions of H-2′ (*δ* 6.44) and H_b-5′ (*δ* 4.70), which were positioned in the 'W'-form. Hence, brachystemidine C was assigned as **3**.

The EIMS, ^1H, ^{13}C NMR, and DEPT spectra (Table 1) indicated that the planar structure of **4** was an 5″-OH-substituted derivative of **1** or **2.** The 5″-OH substituent of **4** was evidenced in the ^{13}C NMR and EIMS spectra. The absence of an OMe signal and a mass of 14 amu less than that of **1** or **2** indicated that the substituent at C-5″ in **4** was an OH rather than an OMe. This change resulted in an upfield shift of the C-5″ resonance from *δ* 87.7 in **1** to *δ* 80.4 in **4**. The clear interaction of H_b-6′ (*δ* 5.10) with C-2′ (*δ* 87.1) in the HMBC spectrum (Table 1) suggested that the 3′-position of **4** was the point of attachment. In addition, the $^1H-^1H$ correlations of H_b-5′ (*δ* 4.76) and H-2′ (*δ* 6.61) showed the typical zigzag coupling. This assumption was verified by X-ray diffraction analysis (Figure 1), which also indicated that the 5″-OH had an α-orientation.

The EIMS and ^1H and ^{13} C NMR spectra (Tables 2 and 3) of **5** were similar to those of **1**−**4**. The main difference in the ^{13}C NMR spectra of **5** compared with **4** was at the pyrrolidone ring. Another carbon signal (*δ* 68.1) bearing an oxygen atom was observed. The $^1H-^1H$ COSY suggested the presence of X−CH−CH_2−CH−X (C-3″−C−C-5″). The EIMS gave a molecular ion peak at *m*/*z* 308, which was 16 amu more than that of **4**. The above data indicated that the planar structure of **5** was closely related to 3″-hydroxybrachystemidine D. A zigzag coupling of H-2′ (*δ*

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Table 1. ^{1}H, ^{13}C NMR and HMBC Data for Compounds **1** and **4**

	1[a]			**4**[b]		
no.	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	HMBC
NH	11.86, br s			10.12, br s		
2		121.5 s			121.8 s	
3	6.62, br s	115.6 d		6.94, m	116.5 d	
4	6.16, br d (2.0)	109.7 d		6.21, dd (6.0, 2.4)	110.6 d	2
5	7.02, d (4.4)	124.4 d		6.94, m	123.8 d	
6		160.0 s			160.7 s	
2′	6.38, br d (3.6)	86.5 d	3′, 4′	6.61, t (2.4)	87.1 d	3′, 4′, 2″, 5″
3′	6.29, br s	127.4 d	6′		134.9 s	
4′		134.3 s		5.97, d (1.2)	126.4 d	2′
5′	4.53 (1H, br d, 13.6, a); 4.73 (1H, br d, 13.6, b)	74.5 t	2′, 4′ 2′, 3′, 4′	4.59 (1H, br d, 13.6, a); 4.76 (1H, dd, 13.6, 2.2, b)	74.9 t	2′, 3′, 4′
6′	4.76 (1H, d, 13.6, a); 4.59 (1H, d, 13.6, b)	58.7 t	3′, 4′, 6	4.67 (1H, br d, 14.8, a); 5.10 (1H, d, 14.8, b)	59.7 t	6, 2′, 3′, 4′
2″		175.1 s			176.2 s	
3″	2.17 (1H, m, a); 2.43 (1H, m, b)	28.8 t	2″, 4″, 5″ 2″, 4″	2.66, (2H, m)	29.3 t	2″, 5″
4″	1.97 (1H, m, a); 1.93 (1H, m, b)	24.4 t	3″ 2″, 5″	1.89 (1H, m, a); 2.25 (1H, m, b)	28.7 t	2″, 5″
5″ OH-5″ OMe	4.74, m; 3.11, s	87.7 d 53.9 q	2′, 2″ 5″	5.37, br s; 4.53, br s	80.4 d	2′, 2″

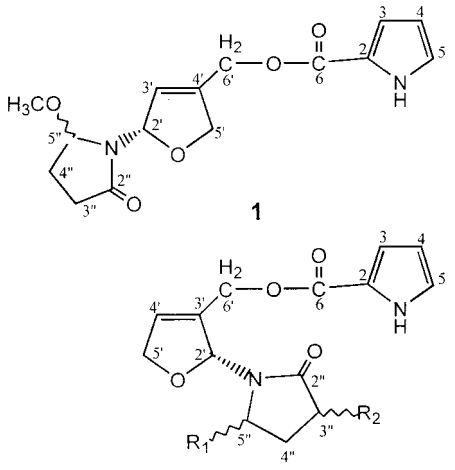
[a] Spectra were recorded in DMSO-d_6 (400 MHz for δ_H and 100.6 MHz for δ_C). [b] Spectra were recorded in $CDCl_3$ (400 MHz for δ_H and 100.6 MHz for δ_C).

Table 2. ^{1}H NMR Data for Compounds **2**, **3**, and **5** (400 MHz)[a]

^{1}H	**2**	**3**	**5**
NH	9.52, br s	9.74, br s	10.50, br s
3	6.92, br d (2.0)	6.90, br d (2.4)	6.97, m
4	6.24, m	6.24, dd (3.2, 1.8)	6.23, dd (2.5, 1.5)
5	6.96, dd (3.8, 2.5)	6.97, br d (1.0)	6.97, m
2′	6.60, br d (4.6)	6.44, d (5.0)	6.55, t (2.0)
4′	6.24, m	6.25, m	6.09, t (1.5)
5′	4.55 (1H, dd, 12.8, 3.2 a); 4.66 (1H, dd, 12.8, 2.2, b)	4.58 (1H, br d, 13.4, a); 4.70 (1H, dd, 13.4, 5.2, b)	4.64 (1H, br d, 14.0, a); 4.74 (1H, dd, 14.0, 1.5, b)
6′	4.73 (1H, dd, 13.6, 2.6, a); 4.71 (1H, d, 13.6, b)	4.76 (1H, d, 13.6, a); 4.97 (1H, d, 13.6, b)	4.69 (1H, d, 14.0, a); 5.02 (1H, d, 14.0, b)
3″	2.25 (1H, m, a); 2.57 (1H, m, b)	4.05, dd (9.0, 5.3)	4.67, m
4″	1.96 (1H, m, a); 1.99 (1H, m, b)	1.87, (1H, m, a); 2.41, (1H, m, b)	2.10, (1H, m, a); 2.39, (1H, m, b)
5″ OH-3″ OH-5″	4.97, m	5.00, dd (6.6, 3.6); 3.61, br s	5.29, d (6.5)
OMe	3.11, s	3.22, s	

[a] Compounds **2, 3** and **5** were all measured in $CDCl_3$.

6.55) and H_b-5′ (δ 4.74) in the ^{1}H−^{1}H COSY spectrum implied that these two protons were coplanar. Thus, the structure of brachystemidine E was assigned as **5**.



1

2 R_1=CH_3O, R_2=H **4** R_1=OH, R_2=H

3 R_1=CH_3O, R_2=OH **5** R_1= R_2=OH

It is noted that alkaloids of this type are seldom found in nature. To our knowledge, the only previous example is 3-furfuryl pyrrole-2-carboxylate, isolated from *Pseudostellaria heterophylla* (Caryophyllaceae)[3] as a natural product.

Table 3. ^{13} C NMR Data for Compounds **2**, **3**, and **5** (100.6 MHz)[a]

^{13}C	**2**	**3**	**5**
2	122.1 s	121.9 s	121.5 s
3	116.0 d	116.0 d	116.5 d
4	110.6 d	110.6 d	110.2 d
5	123.5 d	123.7 d	124.0 d
6	160.2 s	160.8 s	160.9 s
2′	87.5 d	88.3 d	87.1 d
3′	132.0 s	131.5 s	133.9 s
4′	129.7 d	130.7 d	127.4 d
5′	74.4 t	74.8 t	75.0 t
6′	58.8 t	58.7 t	59.3 t
2″	175.9 s	174.8 s	176.7 s
3″	29.3 t	68.6 d	68.1 d
4″	24.1t	32.6 t	38.5 t
5″	88.1 d	86.2 d	77.0 d
OMe-5″	52.1 q	51.8 q	

[a] Compound **2**, **3**, and **5** were all measured in $CDCl_3$.

Experimental Section

General Experimental Procedures. Melting points were determined with a XRC-1 apparatus and are uncorrected. Optical rotations were determined on a JASCO-20C digital polarimeter. Routine ^{1}H (400 MHz) and ^{13}C NMR (100.6 MHz)

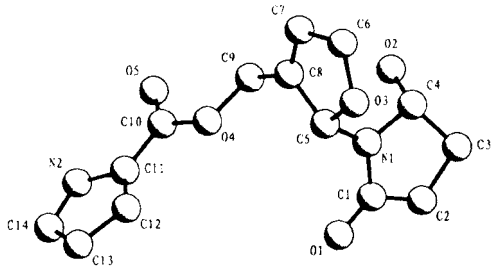


Figure 1. X-ray structure of **4**.

spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal standard. 2D NMR spectra were measured on a DRX-500 spectrometer. MS analyses were carried out on a VG Auto Spec-3000 spectrometer.

Plant Material. The roots (13 kg) of *B. calycinum* were collected in Xishuangbanna of Yunnan Province of China at the end of March, 1999. A voucher specimen (No. 1) was deposited in the herbarium of Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and Isolation. Powdered, dried roots (13 kg) of *B. calycinum* were extracted with 95% EtOH (3 × 50 L) under reflux three times (3 h, 1.5 h, and 1.5 h). After concentration of the combined extracts under reduced pressure, the residues were diluted with H_2O and then partitioned with petroleum ether (60−90 °C), EtOAc, and *n*-BuOH (presaturated with water), respectively. The ethyl acetate portion (50.0 g) was subjected to CC over silica gel (2300 g, 200−300 mesh) eluting with $CHCl_3$−MeOH (17:1 to 8:2, 7 L each eluent) to give five fractions. Fraction 2 was subjected to flash chromatography eluting with petroleum ether−Me_2CO (10:1−5:1) and $CHCl_3$−Me_2CO (10:1−5:1) to afford subfractions 2.1 and 2.2. Fraction 2.1 was chromatographed on Si gel by VLC with $CHCl_3$−EtOAc (5:1−1:1) and $CHCl_3$−Me_2CO (5:1) as eluent to provide **1** (45 mg) and **3** (27 mg). Fraction 2.2 was subjected to Si gel via VLC with $CHCl_3$−Me_2CO (5:1) and $CHCl_3$−^{i}PrOH (10:1−5:1) respectively to furnish **2** (7 mg), **4** (5 mg), and **5** (10 mg).

Brachystemidine A (1): white solid; mp 210−211.5 °C; $[\alpha]_D^{28}$ laevo but unstable (*c* 0.24, MeOH); EIMS *m/z* 306 $[M]^+$ (18), 274 $[M - OMe - H]^+$ (4), 253 (12), 208 (7), 195 (16), 191 (45), 163 (24), 122 (15), 111 (14), 94 (100), 81 (77), 66 (20); ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100.6 MHz) data, see Table 1; HREIMS *m/z* 306.1238 $[M]^+$ (calcd 306.1218).

Brachystemidine B (2): white solid; mp 151−152 °C; $[\alpha]_D^{27}$ −3.1° (*c* 0.32, MeOH); EIMS *m/z* 306 $[M]^+$ (26), 274 $[M - OMe - H]^+$ (17), 207 (4), 195 (31), 192 (55), 191 (59), 181 (9), 180 (10), 163 (45), 135 (23), 122 (31), 111 (34), 94 (100), 81 (88), 71 (58), 66 (41); ^{1}H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Table 3; HREIMS *m/z* 306.1226 $[M]^+$ (calcd 306.1216).

Brachystemidine C (3): colorless gum; $[\alpha]_D^{21}$ −21.0° (*c* 0.25, $CHCl_3$); EIMS *m/z* 322 $[M]^+$ (11), 290 (2), 211 (6), 192 (56), 191 (51), 119 (10), 111 (22), 94 (91), 93 (27), 82 (54), 81 (100), 66 (34); ^{1}H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Table 3; HREIMS *m/z* 322.1176 $[M]^+$ (calcd 322.1165).

Brachystemidine D (4): colorless block; mp 147.5−149 °C; $[\alpha]_D^{25}$ +3.52° (*c* 0.43, MeOH); EIMS *m/z* 292 $[M]^+$ (30), 274 $[M - H_2O]^+$ (6), 192 (63), 191 (71), 182 (35), 181 (47), 163 (52), 135 (32), 111 (48), 94 (100), 81 (95), 66 (60); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Table 1; HREIMS *m/z* 292.1072 $[M]^+$ (calcd 292.1059).

Brachystemidine E (5): colorless gum; $[\alpha]_D^{28}$ +0.76° (*c* 1.65, MeOH); EIMS *m/z* 308 $[M]^+$ (5), 290 $[M - H_2O]^+$ (2), 192 (37), 191 (35), 111 (27), 101 (13), 94 (92), 82 (41), 81 (100), 66 (39); ^{1}H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Table 3; HREIMS *m/z* 308.1022 $[M]^+$ (calcd 308.1008).

Single-Crystal X-ray Analysis of 4.[4] A crystal with the composition $C_{14}H_{16}N_2O_5$ obtained from $CHCl_3$ was used for an X-ray structure determination. Data were acquired with a MAC DIP-2030K diffractometer, Mo Kα radiation (λ 0.71069 Å), graphite monochromator: M_t 292.29 ($C_{14}H_{16}N_2O_5$); crystal size 0.30 × 0.30 × 0.40 mm; monoclinic system, space group $P2_1/c$, 293 K; a = 6.5770(2) Å, b = 10.3520(5) Å, c = 20.5690(9) Å, V = 1400.12 (10 Å^3, D_c = 1.392 g/cm^3, Z = 4. The data were collected at 20 ± 1° by the $\omega-2\theta$ scan technique to a maximum 2θ value of 50.0°. A total of 2280 reflections were collected. The structure was solved by direct methods and expanded by the Fourier technique. The non-H atoms were refined anisotropically; H atoms were included but not refined.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tang, C. L.; Ke, P.; Lu, D. Q.; Zhou, L. H.; Wu, C. Y. *Zhongguo Zhiwu Zhi*; Beijing Science Press: Beijing, 1996; Vol. 26, p 253.
(2) Wu, C. Y.; Zhou, T. Y.; Xiao, P. G. *A Compendium of New China Herbal Medicine*; Shanghai Science and Technology Press: Shanghai, 1990; Vol. 3, p 43.
(3) Reinecke, M. G.; Zhao, Y. Y. *J. Nat. Prod.* **1988**, *51*, 1236−1240.
(4) Crystallographic data for compound **4** has been deposited with the Cambridge Crystallographic Data Center as deposition No. CCDC 163685. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ UK (fax: + 44-(0)1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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