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CUCUBALACTAM AND BRACHYSTEMIN E, TWO NEW COMPOUNDS FROM CARYOPHYLLACEAE

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Abstract -A new pyrrolidone peroxide, named cucubalactam (**1**), and a new octacyclopeptide, named brachystemin E (**2**), have been isolated from Caryophyllaceae plants, *Cucubalus baccifer* and *Brachystemma calycinum*, respectively. Their structures were determined by spectral methods, especially by 1D and 2D NMR techniques. The stereochemistry of compound (**2)** was demonstrated by X-Ray crystallograph.

Caryophyllaceae family covers about 70 genera and about 1750 species, it is distributed world-wide especially in temperate and frigid zones. Many plants in this family such as *Pseudostellaria heterophylla*, *Vaccaria segetalis*, *Dianthus superbus*, *Psammosilene tunicoides* are famous Chinese materia medica. In the past, the chemical investigations on this family focused on saponins,[1] flavonoids,[2] and phytoecdysterones.[3] In recent 10 years, a new kind of metabolites, cyclic peptides, were found in Caryophyllaceae,[4-5] some of them were potent tyrosinase inhibitors.[6-9] We investigated two Caryophyllaceae plants, *Cucubalus baccifer* and *Brachystemma calycinum*, the former has been used for pulmonary tuberculosis and scrofula,[10] and the latter used for rheumatism, limb numbness, impotence and feet edema in China.[11] From the EtOAc extracts of them, two new compounds, cucubalactam (**1**) and brachystemin E (**2**), were isolated using ninhydrin as coloring reagent. We here reported their isolation and structural elucidation.

Cucubalactam (**1**) was isolated as white amorphous powders from the EtOAc extract of *C. baccifer* and tested positive to ninhydrin reagent. The molecular formula $C_{10}H_{17}NO_3$ was derived from HRFABMS at *m/z* 200.1285 $[M+H]^+$ (calcd 200.1287). The ^1H and ^{13}C NMR spectra (Table 1) indicated three methyls, three methylenes, one methine and three quaternary carbons. An amide NH signal (δ 6.99 br s) was observed in ^1H NMR spectrum. The partial structure $-CH_2-CH_2-CH-$ was readily obtained due to ^1H-^1H cross peaks between H-3 (δ 2.42) and H-4 (δ 2.07), H-4 and H-5 (δ 5.33). In the HMBC spectrum (Figure 1), H-3 (δ 2.42, 2.18), H-4 (δ 2.07, 2.24) and H-5 were all correlated with C-2 (δ 178.4), which suggested the presence of 5-substituted pyrrolidone. In addition, observation of ^2J and ^3J connectives of H-11 and H-12 (δ 1.30) to C-8 (δ 83.6), C-9 (δ 148.2), H-13 (δ 1.74) to C-8, C-9 and C-10 (δ 111.4) in the HMBC spectrum indicated another partial structure $CH_2{=}C(CH_3)-C(CH_3)_2-$. C-5 ($\delta$ 88.0) and C-8 (δ 83.6) should bear an oxygen respectively according to their chemical shifts and molecular weight. Optical rotation showed it was racemic. Thus the proposed structure of **1** was determined to be 5ξ-(1,1,2-trimethylallylperoxy)pyrrolidin-2-one.

Table 1. ^1H and ^{13}C NMR Spectral Data of **1** (in $CDCl_3$, 400 MHz for δ_H and 100.6 MHz for δ_C).[a]

position	^1H	^{13}C
1	6.99 br s	
2		178.4 s
3a	2.42 m	28.8 t
3b	2.18 m	
4a	2.24 m	25.4 t
4b	2.07 m	
5	5.33 d (6.6)	88.0 d
8		83.6 s
9		148.2 s
10a	4.88 br s	111.4 t
10b	4.84 d (1.3)	
11	1.30 s	24.4 q[b]
12	1.30 s	24.4 q[b]
13	1.74 s	18.6 q

[a] Coupling constants are presented in Hertz.

[b] Assignments with the same superscripts may be interchanged.

Brachystemin E (**2**) was obtained as crystals from the EtOAc extract of *B. calycinum*. It gave a negative reaction to ninhydrin reagent but positive after hydrolysis with 6mol/L HCl. The molecular formula $C_{45}H_{66}N_{10}O_9$ was derived from the combination of FABMS at *m/z* 891 $[M+H]^+$ and ^{13}C NMR and DEPT spectra, which indicated eighteen degrees of unsaturation. IR absorptions at 3298 and 1651 cm^{-1} were characterized as amide NH and amide carbonyl groups, respectively. Amino acid analysis gave the composition of two prolines, one leucine, two isoleucines, one glycine, one tryptophan and one asparagine, The above evidence suggested **2** was a cyclopeptide.[12] The individual amino acid residues ach-

Table 2. ^{1}H and ^{13}C NMR Spectral Data of **2**(in pyridine-d_5, 400 MHz for δ_H and 100.6 MHz for δ_C)

	CO	α	β	γ	δ	H_N	α	β	γ	δ
Pro^1	175.8 s	60.0 d	29.9 t	26.5 t	47.6 t		4.56 t (7.9)	0.60 m 1.44 m	4.02 m 3.41 m	3.70 m 3.42 m
Leu	171.8 s	61.6 d	40.5 t	25.8 d	22.9 q 21.7 q	9.56 br s	4.19 dd (4.2, 2.5)	2.00 m	1.45 m	0.84 d (6.1) 0.79 d (7.0)
Ile^1	171.4 s	58.0 d	37.3 d	16.1 q 29.9 t	12.0 q	7.98 d (10.4)	5.22 d (4.7)	2.59 m	0.96 d (4.0) 1.44 m	0.98 t (4.6)
Gly	168.0 s	44.7 t				7.92 m	4.99 m 3.59 m			
Pro^2	174.1 s	63.2 d	30.6 t	25.2 t	48.0 t		4.76 t (8.0)	2.33 m 1.93 m	2.14 m 1.78 m	4.12 m 3.62 m
Ile^2	173.6 s	55.5 d	36.6 d	15.8 q 25.4 t	12.0 q	7.76 d (5.5)	4.58 m	1.86 m	1.04 d (6.8) 1.90 m 1.35 m	0.76 d (5.7)
Trp	173.3 s	55.1 d	26.7 t	109.2 s	125.0 d 128.5 s 119.2 d 122.5 d 120.0 d 112.5 d 137.9 s	6.75 br s	5.20 m	4.00 m 3.39 m		7.48 d (2.2) 12.40 br s 7.92 m 7.22 m 7.34 m 7.64 m
Asn	170.5 s	50.1 d	35.7 d	175.5 s		7.74 m	5.25 d (4.6)	3.10 dd (13.9, 6.6) 3.58 dd (13.9, 6.6)		7.91 m (NH_2)

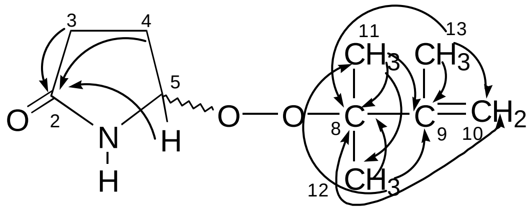

Figure 1 Major HMBC correlations (H→C) of **1**

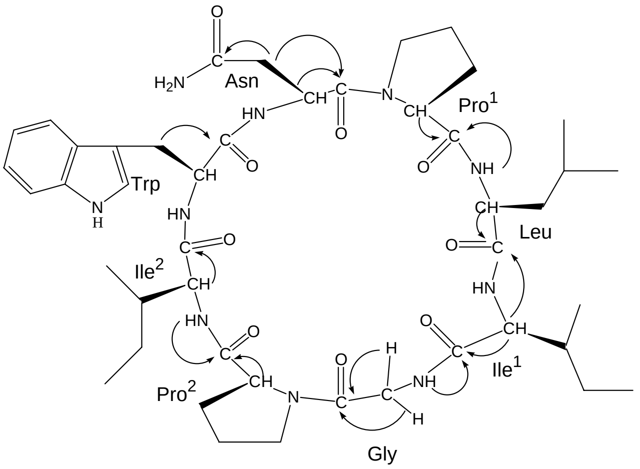

Figure 2 Selective HMBC correlations (H→C) of **2**

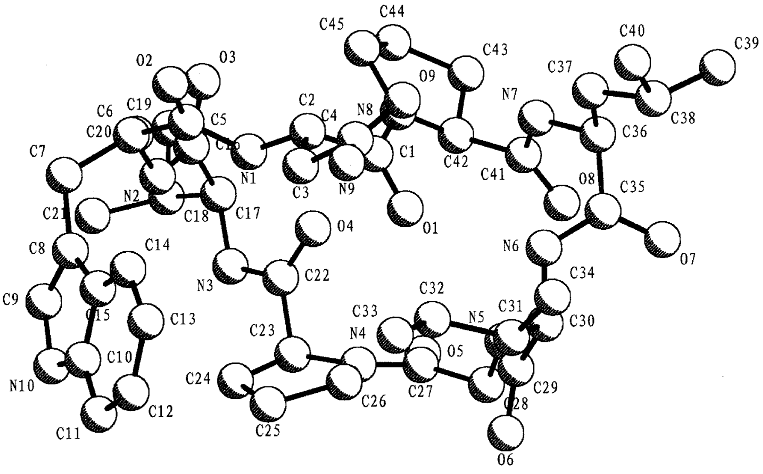

Figure 3 The X-Ray plot of **2**

ieved from ^{1}H-^{1}H COSY and TOCSY spectra were identical with those of amino acid analysis. The sequence of these amino acids were carried out by HMBC (Figure 2) and X-Ray diffraction analysis (Figure 3). Observation of ^2J and ^3J connectivities between Gly$_{\mathrm{NH}}$ (δ 7.92) and Ile$^1_{\mathrm{CO}}$ (δ 171.4), Ile$^1_{\alpha\mathrm{H}}$ (δ 5.22) and Leu$_{\mathrm{CO}}$ (δ 171.8), Leu$_{\mathrm{NH}}$ (δ 9.56) and Pro$^1_{\mathrm{CO}}$ (δ 175.8), Ile$^2_{\mathrm{NH}}$ (δ 7.76) and Pro$^2_{\mathrm{CO}}$ (δ 174.1) in the HMBC spectrum suggested two peptide fragments -Pro^1-Leu-Ile^1-Gly-CO- and -Pro^2-Ile^2-CO- existed. The remaining linkage was solved by X-Ray diffraction analysis. Thus the cyclic peptide was determined as cyclo(Pro^1-Leu-Ile^1-Gly-Pro^2-Ile^2-Trp-Asn). The ^{1}H and ^{13}C NMR signals were assigned by using ^{1}H-^{1}H COSY, TOCSY, HMQC and HMBC spectra (Table 2). The X-Ray diffraction analysis suggested all amino acids were L- or D-form.

It was noted that the chemical shifts of γ-protons of proline ^{1} are shifted to very downfield, this is due to the dishielding effect of carbonyl group of asparagine.

Pharmacological tests of **1** and **2** were taken on using cardiovascular (PP1), anti-cancer (CDC25), antibacterial (PEPT) and antifungal (YNG) bioassay, no biological activities were observed at concentration of 61 μg/mL, 25 μg/mL, 96 μg/mL and 4 μg/mL respectively.

EXPERIMENTAL

General Experimental Procedures. Melting points were recorded on a XRC-1 apparatus and were uncorrected. Optical rotation was determined on a JASCO-20C digital polarimeter. IR spectrum were measured on a Bio-Rad FTS-135 spectrophotometer. Amino acid analysis was performed on a Hitachi 835-50 amino acid analyzer. FABMS and EIMS were determined on a VG Auto Spec-3000 spectrometer. Regular ^{1}H and ^{13}C NMR spectra were run on a Bruker AM-400 spectrometer, 2D NMR experiments were carried out on a DRX-500 spectrometer.

Plant Material. The whole plants of *C. baccifer* were collected in Chenggong county of Yunnan province of China in September 1999 and identified by Dr. Shui YM. The roots of *B. calycinum* were collected in Xishuangbanna of Yunnan province of China in March 1999 and identified by Prof. X. Zhuang. The voucher specimen were both deposited in the herbarium of Kunming Institute of Botany.

Extraction and Isolation. The dried whole plants of *C. baccifer* (24.0 kg) were extracted with 95% EtOH (3×90 L) under reflux for three times (2 h, 1 h and 1 h respectively). After concentration of the combined extracts in *vacuum*, the residues (700.0 g) was suspended in water and then partitioned with petroleum ether (bp 60-90 ^{0}C), EtOAc and *n*-BuOH, respectively. The EtOAc fraction (400.0 g) was decolorized on Diaion HP 20 eluted with gradient MeOH-H_2O (0:1-1:0). The 70% MeOH eluate (250.0 g) was subsequently subjected to column chromatography over silica gel (2500g, 200-300 mesh) eluted with $CHCl_3$-MeOH (48:1 to 8:2, 6500 mL each eluent) to give six fractions. Fraction 5 (3.0 g) was chromatographed on silica gel eluted with $CHCl_3$-Me_2CO (20:1-5:1, 1000 mL each eluent) to obtained subfraction (200 mg), which was further purified on silica gel (10-40 μm) using increased polarity of $CHCl_3$-i-PrOH (15:1-5:1) as eluent to furnish cucubalactam (**1**)(30 mg).

The powered roots of *B. calycinum* (13.0 kg) were extracted with 95% EtOH (3×50 L) under reflux for three times (3 h, 1.5 h and 1.5 h respectively). After concentration of the combined extracts under reduced pressure, the residue was diluted with water and then partitioned with petroleum ether, EtOAc and *n*-BuOH (presaturated by water) respectively. The EtOAc portion (50.0 g) was subjected to column chromatography over silica gel (2300 g, 200-300 mesh) eluted with $CHCl_3$-MeOH (17:1 to 8:2, 7000 mL each eluent) to give five fractions. Fraction 5 (2.0 g) was chromatographed on silica gel eluted with $CHCl_3$-MeOH-H_2O(9:1:0 to 8:2:0.2, 1500 mL each eluent) to afford fraction 5.1 (127 mg) which was further purified on RP-18 using stepwise gradient MeOH-H_2O (45-70%) as eluent to yield brachystemin E (**2**) (59 mg).

Cucubalactam (**1**) $C_{10}H_{17}NO_3$: white amorphous powders; mp 32-33 ^{0}C ($CHCl_3$); $[\alpha]_D^{28}$ 0^0 (*c* 0.32 MeOH); FABMS *m/z* 200 $[M+H]^+$ (100); HRFABMS *m/z* 200.1285 $[M+H]^+$, (calcd 200.1287); EIMS *m/z* 200 $[M+H]^+$ (6), 117 (6), 97 (25), 84 (100); ^{1}H and ^{13}C NMR data see Table 1.

Brachystemin E (**2**) $C_{45}H_{66}N_{10}O_9$: crystals; mp 210-211.5 ^{0}C (MeOH), $[\alpha]_D^{25.1}$ -48.5^O (*c* 0.52, MeOH); IR (KBr) ν_{max} 3298, 1651 cm^{-1}; FABMS *m/z* 891 $[M+H]^+$ (100), 760, 706, 606, 493, 412, 324, 283, 226, 155, 99; ^{1}H and ^{13}C NMR data see Table 2.

Amino acid analysis of **2**. Amino acid analysis to the complete hydrolysates prepaired by treating **2** with 6 mol/L HCl at 110 ^{0}C for 24 h in a sealed tube indicated the presence of Pro (2eq.), Leu (1eq.), Ile (2eq.), Gly (1eq.), Trp (1eq.) and Asn (1eq.).

X-Ray analysis of **2**. **2** was crystallized from MeOH as quadrate crystals, triclinic system; space group *P1*, unit cell dimentions: a=9.984 (1), b=10.446 (1), c=16.011 (2) Å; α=71.788 (9)^0, β=80.045 (7)^0, γ=71.606 (9)^0, V=1500.0 (3) Å^3; Z=1; D_{calc}=1.173g/cm^3; R_f =0.056, R_w=0.060 ($w=1/\sigma^2|F|$), $(\Delta/\sigma)_{max}$=0.185, $(\Delta\rho)_{min}$=-0.730e/ Å^3, $(\Delta\rho)_{max}$=1.380e/ Å^3; Observed reflections: 3397.

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REFERENCES AND NOTES

1. S. P. S. Bhandari, P. K. Agrawal, and H. S. Garg, *Phytochemistry*, 1990, **29**, 3889.
2. Z. T. Ding, J. Zhou, and N. H. Tan, *Planta Med.* 1999, **65**, 578.
3. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, *Chem. Nat. Compd.*, 1993, **29**, 551.
4. M. S. Liu, Y. J. Chen, Y. H. Wang, S. R. Xing, M. Tokido, and K. Yasukawa, *Acta Pharmaceutica Sinica*, 1992, **27**, 667.
5. Y. X. Cheng, J. Zhou, and N. H. Tan, *Acta Botanica Sinica*, 2001, **43**, 760.
6. H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya, and H. Itokawa, *Tetrahedron*, 1994, **50**, 6797.
7. H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya, and H. Itokawa, *Tetrahedron*, 1994, **50**, 9975.
8. H. Morita, H. Kobata, K. Takeya, and H. Itokawa, *Tetrahedron Lett.*, 1994, **35**, 3563.
9. H. Morita, T. Kayashita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1995, **58**, 943.

10.Institute of Botany of Academia Sinica, ‘*Zhongguo Gaodeng Zhiwu Tujian*’, Science and Technology Press, Beijing, 1980, p. 642.

11. C. Y. Wu, T. Y. Zhou, and P. G. Xiao, *A Compendium of New China Herbal Medicine*, Shanghai Science and Technology Press, Shanghai, 1990, **3**, 43.

12. J. Zhou, and N. H. Tan, *Chinese Science Bulletin*, 2000, **45**, 1825.