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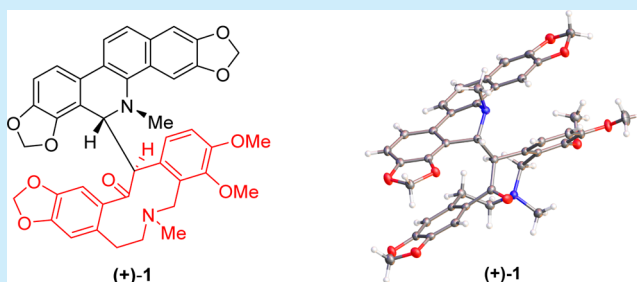
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Two Pairs of Enantiomeric Alkaloid Dimers from *Macleaya cordata*Chun-Mei Sai,[†] Da-Hong Li,[†] Chun-Mei Xue,[†] Kai-Bo Wang,[†] Ping Hu,[†] Yue-Hu Pei,[†] Jiao Bai,[†] Yong-Kui Jing,[‡] Zhan-Lin Li,^{*,†} and Hui-Ming Hua^{*,†}[†]Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning, P. R. China[‡]Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, United States

S Supporting Information

ABSTRACT: Two pairs of enantiomeric alkaloid dimers, (±)-macleayins A (**1**) and B (**2**), representing a novel dimerization pattern of two different types of alkaloids via a C–C σ -bond, were isolated from the aerial parts of *Macleaya cordata*. The enantiomeric separation was achieved by chiral chromatography. Their structures and stereochemistry were determined by the analysis of extensive spectroscopic data, electronic circular dichroism calculation, and single-crystal X-ray diffraction data. (–)-Macleayin A exhibits modest cytotoxic activity against HL-60 cell line with the IC₅₀ value of 3.51 μ M.



Macleaya cordata (Willd.) R. Br., belonging to family Papaveraceae, is a perennial plant, which is mainly distributed in the northwest and southwest parts of China, Southeast Asia, North America, and Europe.¹ As a traditional folk herb in China, it has been used for over 1000 years as an ordinary medication to relieve muscle pain and to treat inflamed wounds and bee bites. Currently, it is utilized for the treatment of incised wound, arthritis, rheumatism, arthralgia, and trichomonas vaginalis.² Moreover, it has been widely used to treat cervical cancer and thyroid cancer in China, North America, and Europe.³ Notably, *M. cordata* has been extensively used not only in human medicines but also in stockbreeding and agriculture.⁴ For example, *M. cordata* is on the European Food Safety Authority list of plants exploited as a component in feed additives in animal production.^{2,4}

As reported previously, alkaloids including benzophenanthridines, protopines, and protoberberines were considered as major bioactive constituents of *M. cordata*,² which exhibited antibacterial,^{5a} antifungal,^{5b} anti-inflammatory,^{5c} insecticidal,^{5d} anticancer,^{5e} and animal growth promotion activities.^{5f} In light of their intriguing structures and significant biological activities, those families of alkaloids have attracted attention broadly from the scientific communities in recent decades. In our continuing search for structurally unique molecules with significant antitumor activity from traditional Chinese medicines,⁶ the chemical constituents of *M. cordata* have been investigated in depth. As a result, two pairs of novel enantiomeric natural alkaloid dimers, (±)-macleayins A (**1**) and B (**2**), were isolated, which represented the first dimeric alkaloids arising from the conjugation between benzophenanthridine and protopine moieties. The 6,13'-coupling pattern hints a hitherto unprecedented C–C linkage in this type of dimer, leading to two chiral centers. Herein, the isolation, structure elucidation, chiral resolution, stereochemical assignment, and cytotoxic activity, as

well as the plausible biosynthetic pathway of compounds **1** and **2** are described.

Macleayin A (**1**), initially obtained as a white powder, had a molecular formula C₄₁H₃₆N₂O₉ with 25 degrees of unsaturation as established by HRESIMS at *m/z* 701.2489 [M + H]⁺ (calcd 701.2494). Its IR spectrum exhibited characteristic absorption bands of ketone group (1668 cm^{−1}), methylenedioxy group (2792, 939 cm^{−1}), and aromatic ring (1619, 1485, 1463 cm^{−1}), respectively. Its UV spectrum had maximum absorptions at 230 and 288 nm. The ¹H NMR (Table 1) spectrum showed three AB spin systems of aromatic protons in *ortho*-position, two aromatic protons in *para*-position, one aromatic proton in singlet, as well as those of three methylenedioxy groups, two methoxyl groups, and two *N*-methyl groups. The above data suggested that **1** might be a dimeric alkaloid, supported by its ¹³C NMR and HRESIMS data. The ¹³C NMR (Table 1) and HSQC spectra resolved 40 carbons, including twenty-eight aromatic, three methylenedioxy, two methoxyls, two *N*-methyls, three methylenes, and two sp³ methine carbons (Table 1). In addition, according to its molecular formula, one carbon signal was not displayed.

The presence of sanguinarine (subunit C, Figure 2) in **1** was supported by the ¹H NMR signals for two pairs of *ortho*-coupled protons at δ_{H} 7.68 (H-11) and 7.46 (H-12), together with 7.12 (H-10) and 6.57 (H-9), two aromatic singlets at δ_{H} 7.01 (H-1) and 6.61 (H-4), two methylenedioxy groups at δ_{H} 6.11, 5.95, 5.91 and 5.89, and one *N*-methyl at δ_{H} 2.49, and their corresponding ¹³C NMR signals. The observed HMBC correlations, from H-1 to C-3, C-4a, and C-12, from H-4 to C-2, C-4b, and C-12a, from H-10 to C-6a, from H-11 to C-4b, C-10a, and C-12a, from H-12 to C-1, C-4a, and C-10b, from 5-NCH₃ to C-6 and C-4b, and from two

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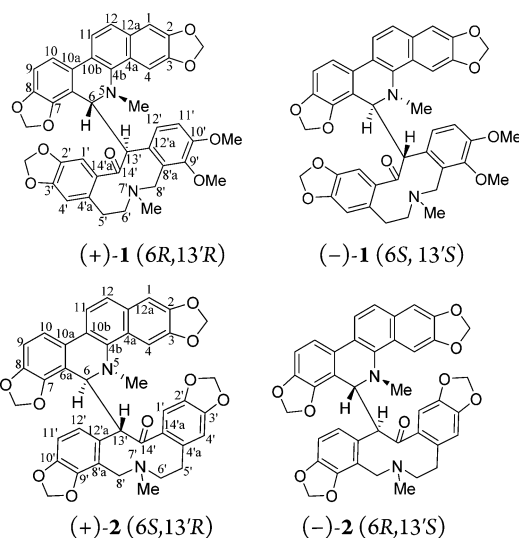
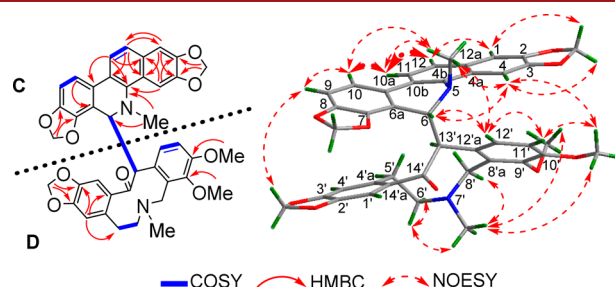
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Table 1. ^1H (600 MHz) and ^{13}C NMR (100 MHz) Data for **1** and **2** in CDCl_3 (δ ppm)

no.	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	7.01 (s)	104.0	7.01 (s)	104.0
2		147.5		146.6
3		147.8		147.5
4	6.61 (s)	100.8	5.35 (brs)	101.0
4a		127.8		127.3
4b		139.2		139.5
6	4.81 (d, 9.3)	57.3	<i>a</i>	<i>a</i>
6a		115.3		114.6
7		144.8		145.1
8		147.0		146.9
9	6.57 (brs)	107.2	6.72 (d, 8.0)	107.8
10	7.12 (brd, 7.9)	116.6	7.21 (d, 8.0)	116.6
10a		125.6		125.9
10b		124.5		123.8
11	7.68 (d, 8.5)	119.8	7.74 (d, 8.3)	120.0
12	7.46 (d, 8.5)	124.0	7.49 (d, 8.3)	124.3
12a		130.8		130.8
1'	<i>a</i>	111.6	<i>a</i>	110.0
2'		145.6		145.9
3'		148.6		146.1
4'	6.23 (s)	110.2	5.92 (brs)	110.2
4'a		130.4		<i>a</i>
5'	2.56 (brs)	33.9	2.30–1.65 (m)	<i>a</i>
	1.96 (d, 15.0)			
6'	2.39 (d, 10.3)	57.3	2.30–1.65 (m)	<i>a</i>
	1.81 (brs)			
8'	3.07 (d, 13.2)	48.4	3.22 (brs)	<i>a</i>
	2.32 (brs)		2.98 (brs)	
8'a		130.8		<i>a</i>
9'		147.0		147.2
10'		150.6		150.1
11'	7.07 (brd, 8.1)	110.8	6.78 (d, 6.6)	106.7
12'	7.50 (brs)	125.4	7.79 (brs)	124.05
12'a		131.5		<i>a</i>
13'	4.51 (brs)	52.7	5.05 (brs)	64.4
14'		<i>a</i>		<i>a</i>
14'a		135.1		<i>a</i>
5-NCH ₃	2.49 (s)	40.9	2.57 (s)	42.0
7'-NCH ₃	1.52 (s)	41.6	1.87 (s)	43.2
2,3-OCH ₂ O	5.91 (d, 1.2)	101.4	5.99–5.83 (m)	101.0
	5.89 (d, 1.2)			
7,8-OCH ₂ O	6.11 (d, 1.5)	100.9	5.99–5.83 (m)	101.1
	5.95 (brs)			
2',3'-OCH ₂ O	5.92 (d, 1.4)	101.7	5.99–5.83 (m)	100.6
	5.90 (d, 1.4)			
9', 10'-OCH ₃ or OCH ₂ O	3.46 (s)	60.9	5.99–5.83 (m)	101.6
	3.95 (s)	56.1		

*a*No signal observed in ^1H NMR and ^{13}C NMR spectra.

methylenedioxy proton signals to C-2, C-3, C-7, and C-8, as well as the proton spin systems of H-9/H-10 and H-11/H-12 revealed by the ^1H – ^1H COSY experiment (Figure 2), further confirmed the structure deduced above. Moreover, the fragment peak at m/z 332.0969 in the HRESIMS/MS, an immonium ion produced

Figure 1. Structures of (\pm)-**1** and **2**.Figure 2. ^1H – ^1H COSY, HMBC, and NOESY correlations for **1**.

by cleavage of C6–C13' single bond, supported the assignment of subunit C.⁷

The structure moiety of allocryptopine (subunit D, Figure 2) was elucidated by comparison of the NMR and MS data with those reported.⁸ The ^1H NMR signals including three aromatic protons, one methylenedioxy, two methoxys, and three methylenes, and the corresponding ^{13}C NMR signals suggested the presence of subunit D. The carbonyl carbon of C-14' was not observed in ^{13}C NMR spectrum, which was in accordance with that reported for allocryptopine.⁸ Finally, the linkage of subunits C and D via C-6 and C-13' was established by the COSY correlation of H-6 and H-13'. The planar structure of **1** was thus determined as depicted.

Due to the missing of a key carbon signal (C-14'), a proton signal (H-1'), and some HMBC correlations, the gross structure of **1** could not be established unambiguously on the basis of the existing 1D and 2D NMR data. Fortunately, a crystal suitable for X-ray crystallographic study was obtained upon slow evaporation of the solvent mixture (CH_2Cl_2 –PhMe–*n*-hexane) by keeping the sample at room temperature for 15 days. The final refinement on the Cu K α data resulted in a Flack parameter of *N*, and the crystal of **1** had a $p2_1/c$ space group, indicating a racemic nature, which was in accordance with the lack of optical activity.^{9,10} Furthermore, the X-ray diffraction analysis (Figure 3) allowed to unambiguously assign the absolute configurations of the two enantiomers of **1** to be (6*R*,13'*R*) and (6*S*',13'*S*'), respectively (SI). Subsequent chiral resolution of **1** was performed on a chiral column to yield (+)-**1** and (–)-**1**, which were virtually opposite in terms of their CD curves (Figure 4) and optical rotation data ($[\alpha]_{\text{D}}^{20}$ (c 0.05 MeOH) + 254 (**1a**) and $[\alpha]_{\text{D}}^{20}$ (c 0.05 MeOH)

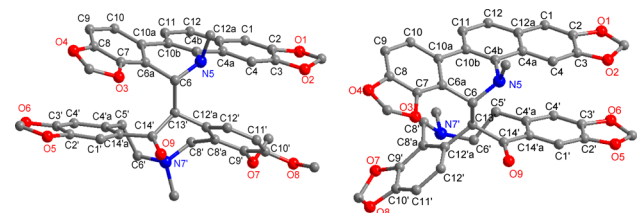


Figure 3. Diamond plot of X-ray crystallographic data for (±)-1 and (±)-2.

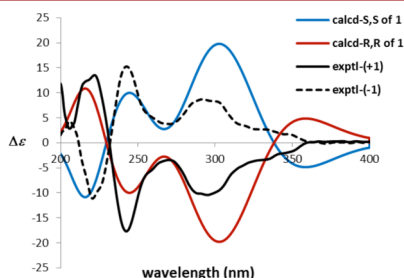


Figure 4. Experimental and suitable calculated ECD spectra of (±)-1.

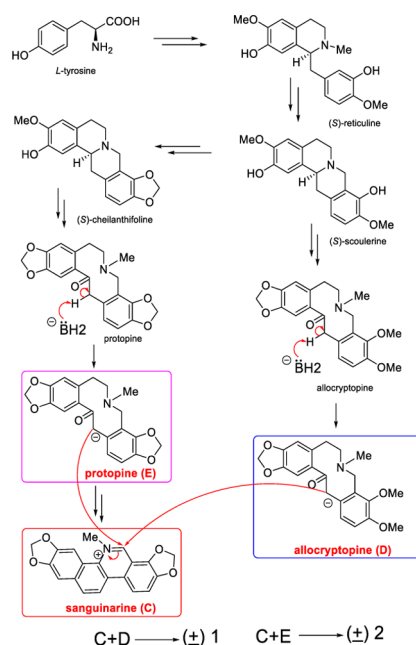
– 230 (1b). The final assignment of (+)-1 (6*R*,13'*R*) and (–)-1 (6*S*,13'*S*) was made by the comparison of the calculated electronic circular dichroisms (ECD) via a quantum method with the experimental data (Figure 4).

Macleayin B (2) was obtained as a white powder with a quasi-molecular ion peak at m/z 685.2172 [$M + H$]⁺ (calcd 685.2181) in HRESIMS, coinciding with the molecular formula C₄₀H₃₂N₂O₉). A comparison of ¹H and ¹³C NMR data (Table 1) of 2 with those of 1 showed that they were structural analogues. The only difference is the presence of one more methylenedioxy and the absence of two methoxys in 2, suggesting that compound 2 contains two structural moieties of sanguinarine and protopine. In addition, the moiety of sanguinarine was further confirmed by the analysis of its HRESIMS/MS (m/z 332.0952). However, due to the deficiency of many signals in subunit E (protopine), the exact partial structure could not be established on the basis of the 2D NMR. An X-ray crystallographic experiment (Figure 3) explicitly confirmed the structure of (±)-2 with configurations of (6*S*,13'*R*) and (6*R**,13'*S**) (SI). Separation by using chiral-phase HPLC yielded (+)-2 ($[\alpha]_D^{20}$ (c 0.07 MeOH) + 41) and (–)-2 ($[\alpha]_D^{20}$ (c 0.07 MeOH) – 43) in a ratio of 1:1, whose absolute configurations were established by comparing the calculated ECD spectra with the experimental spectra (SI). From the above evidence, the absolute stereochemistry for (+)-2 (6*S*,13'*R*) and (–)-2 (6*R*,13'*S*) were unambiguously determined as shown in Figure 1. It was noteworthy that several carbon signals of protopine moiety of 2 were not observed in the ¹³C NMR spectrum, which remained unaccountable.

Although the discovery of macleayins A and B is of great interest, it raises a question whether 1 and 2 are natural products or artifacts. The crude ethanol extracts by cold maceration and reflux were analyzed by LC–MS, which distinctly exhibited corresponding chromatographic peaks (SI) with quasi-molecular ion peaks at m/z 701, 685 and fragment peaks at m/z 332 consistent with those of compounds 1 and 2, demonstrating the natural occurrence of 1 and 2.

Hypothetical biosynthetic pathways for compounds 1 and 2 were proposed (Scheme 1). Sanguinarine (C), allocryptopine (D), and protopine (E) were considered as the biogenetic

Scheme 1. Plausible Biogenetic Pathways for Compounds 1 and 2



intermediates of compounds 1 and 2. The biogenesis of sanguinarine and allocryptopine started with the condensation of two tyrosine derivatives, subsequently by a series of reactions to produce (S)-reticuline derivatives.¹¹ The reaction from (S)-reticuline to sanguinarine was catalyzed by berberine bridge enzyme (BBE) to form (S)-scoulerine, which represented the first committed step in the branch pathway. Subsequently, (S)-cheilanthifoline was converted to protopine and sanguinarine by a series of synthase catalysis. Allocryptopine was biosynthesized from another pathway initiated from the (S)-scoulerine, which is similar to protopine.^{1b} Compounds 1 and 2 were finally formed by the nucleophilic substitution reaction between C and D or E. The racemization of these two compounds may be an enzyme-catalyzed reaction, for that the single enzyme lack of stereospecificity, which generates both enantiomers, had been reported,¹² and the subunits C, D, and E without chiral center have nearly planar structure. However, there is another possibility that the spontaneous nucleophilic attack in plant cells results in the formation of a pair of enantiomers in a ratio of 1:1. However, the mystery of racemization needs to be disclosed by the synthetic and biological efforts.

Compounds 1, 2, (+)-1, (–)-1, (+)-2, and (–)-2 and their biogenetic monomeric precursors sanguinarine, allocryptopine, and protopine were evaluated for in vitro antiproliferative activities against three human cancer cell lines, HL-60, MCF-7 and A-549, using the trypan blue method and MTT method^{13a,b} reported previously, and 5-fluorouracil was used as positive control. Compounds 1 and 2 exhibited more potent cancer cell growth inhibitory activities against HL-60 cell lines than their biogenetic monomeric precursors. Notably, (–)-1 and (–)-2 showed more modest activity than those of (+)-1 and (+)-2 (Table 2).

Dimeric natural products are a special class of molecules frequently possessing complex structure and significant bioactivities.¹⁴ In this contribution, (±)-macleayins A (1) and B (2) represent a new carbon skeleton formed by involving an unusual dimerization pattern of two different types of alkaloids via carbon

Table 2. In Vitro Cytotoxic Activities against HL-60, A-549, and MCF-7 Cancer Cell Lines

compsds	HL-60 IC ₅₀ (μM)	A-549 IC ₅₀ (μM)	MCF-7 IC ₅₀ (μM)
1	2.65	12.45	10.87
2	5.58	34.87	49.68
(+)-1	5.62		
(-)-1	3.51		
(+)-2	9.64		
(-)-2	8.16		
sanguinarine	7.71	8.49	5.51
alocryptopine	7.18	26.06	28.24
protopine	8.94	27.37	25.19
5-Fu	2.80	1.60	17.01

bond. Moreover, these novel structures showed modest cytotoxic effects against a series of cancer cells and might give some insight into new lead ligands for the development of anticancer drugs, while further investigations such as synthetic effort and in-depth biological testing are really needed.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02044.

X-ray crystal details for **1** (CIF)

X-ray crystal details for **2** (CIF)

Experimental procedures, 1D and 2D NMR, HRESIMS, CD, UV IR spectra, X-ray crystal structure, and details of the quantum chemical ECD calculations for compounds **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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