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Novel cyclopeptide and unique flavone from *Desmos rostrata*. Total synthesis of desmorostratone

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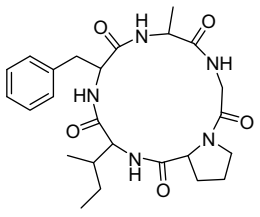
ABSTRACT

Two new compounds, a cyclic peptide desmocyclopeptide (**1**) and a special flavone desmorostratone (**2**) were isolated from the stem bark of *Desmos rostrata*, along with two known compounds, desmosdumotins B (**3**) and C (**4**). Their structures were established on the basis of the spectral data, including mass spectrometry and 2D NMR. The total synthesis of desmorostratone (**2**) was performed in order to confirm its structure as well as that of desmosdumotin C (**4**), which was a tautomeric mixture in the solution. Finally, cytotoxity of these compounds were evaluated. Desmosdumotin C (**4**) displayed a moderate inhibition activity against KB cell line with an IC_{50} of 19.2 μM, whereas the other products showed a weak inhibition against the same cell line target.

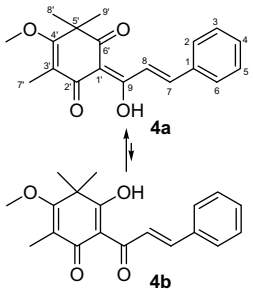
1. Introduction

In the course of a screening program on the flora of Vietnam, the plant *Desmos rostrata* P.T. Li (basionym: *Dasymaschalon rostratum*, Annonaceae) was selected for its cytotoxicity against KB cell line (15% inhibition at the concentration of 1 μg/mL for the stem bark extract). We have previously isolated and characterized from this plant several antiplasmodial alkaloids, containing the tetrahydroisoquinoline motif.[1] Analysis of the CH_2Cl_2 fraction of the stem bark of *D. rostrata* led to the isolation of two new compounds, a cyclic peptide desmocyclopeptide (**1**) and an unique flavone type desmorostratone (**2**), together with two known compounds, desmosdumotins B (**3**)[2] and C (**4**).[3a,b] Cyclopeptide compounds are usually found in the seed of the Annonaceae plants and rarely observed from stem bark. Desmosdumotin C (**4**) has been previously reported from *Desmos dumosus* and *Campomanesia lineatifolia* as a single tautomer, whereas it was isolated from *D. rostrata* in a tautomeric mixture of the two isomers as indicated by its ^1H NMR spectrum.

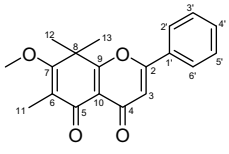
In order to confirm the structures of desmorostratone (**2**) and desmosdumotin C (**4**), their total syntheses were attempted, starting from 1-acetyl-2,4,6-trihydroxybenzene. Cytotoxicity of these compounds was evaluated on KB cells. However, they showed a moderate or weak cytotoxicity.



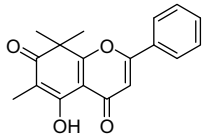
desmocyclopeptide (**1**)



desmosdumotin C (**4**)



desmorostratone (**2**)



desmosdumotin B (**3**)

2. Results and discussion

Compound **1** was obtained as white solid (mp 261–262 °C) and optically active $[\alpha]_D^{20}$ −126.7 (*c* 2, $CHCl_3$). In its HR-ESI mass spectrum, the protonated molecular ion was observed at *m/z* 486.2727 $[M+H]^+$ (calcd 486.2716 for $C_{25}H_{36}N_5O_5$), suggesting the molecular formula $C_{25}H_{35}N_5O_5$. The peptide nature of **1** was suggested by the

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molecular formula itself and from elucidation of its NMR spectra. Analysis of the ^{1}H and ^{13}C NMR spectral data for **1**, with the aid of ^{1}H–^{1}H-COSY, HSQC, HMBC and NOESY spectra, indicated the presence of an alanine, a glycine, a proline, an isoleucine and a phenylalanine (Table 1). The determination of the peptide sequence was established from the connectivity of the carbonyl of residue (*i*) and the NH proton of residue (*i*+1) on the HMBC spectrum as follows: the correlations of the carbonyl at δ_C 173.5 of the Ala[1] with its Hα proton at δ_H 4.37, as well as to the NH proton of the Gly[2] at δ_H 7.78 were observed. The carbonyl group of the Gly[2] at δ_C 168.5 was not correlated to an amide proton, suggesting its connection with the Pro[3]. Cross-peaks of the carbonyl of the Pro[3] at δ_C 172.1 with its Hα proton at δ_H 4.21 and NH proton (δ_H 7.12) of the Ile[4] were presented. In addition, the carbonyl of Ile[4] at δ_C 172.3 was correlated with its Hα proton at δ_H 4.18 and NH proton of Phe[5] at δ_H 7.56. Finally, ^{2}J-HMBC correlation of the carbonyl of Phe[5] at δ_C 172.3 with its Hα proton (δ_H 4.64) and NH proton of Ala[1] (δ_H 8.25) were noted. Complete analysis of the HMBC spectrum determined the structure of **1** as drawn in Figure 1. The NOESY spectrum of **1** depicted the strong spatial interactions of Hα of Gly[2] with Hδ of Pro[3], indicating that the amide bond between Gly[2]–Pro[3] was trans.[4] The $d_{NN(i,i+1)}$ and $d_{\alpha N(i,i+1)}$ sequential connectivity from Ala[1] to Phe[5] was clearly observed in the NOESY spectrum as indicated in Figure 1. The amino acids residue and the sequence of **1** were confirmed by fragmentation of $[M+H]^+$ ion at *m*/*z* 486. A series of adjacent a_n ion peaks were observed at *m*/*z* 311, 240, 183 and 86 corresponding to the successive loss of Phe, Ala, Gly and Pro. A second series of peaks at *m*/*z* 339, 268, 211 and 114 were assigned to adjacent b_n ion (loss of Phe, Ala, Gly and Pro). This new pentacyclopeptide **1** is named desmocyclopeptide. The structure of **1** is very close to that of pseudostellarin A which has been previously reported from the roots of *Pseudostellaria heterophylla*.[5]

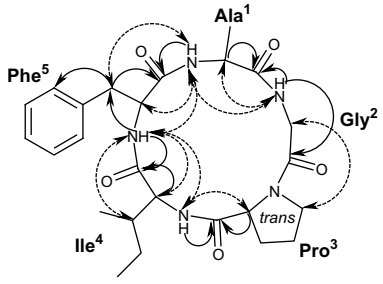


Figure 1. Selected HMBC (→) and NOESY (↔) correlations of **1**.

The absolute configuration of the chiral amino acids in desmocyclopeptide (**1**) was determined by HPLC analysis of the acid hydrolysate derivatised with Marfey's reagent,[6] and comparison with appropriate amino acid standards, that secured the L-configuration for Ala, Ile, Phe and Pro.

Compound **2** was isolated as yellow solid (mp 199–201 °C). The HR-ESIMS of **2** showed the pseudomolecular ion $[M+H]^+$ at *m*/*z* 311.1279 (calcd 311.1283 for $C_{19}H_{19}O_4$), suggesting the molecular formula $C_{19}H_{18}O_4$. This indicated eleven degrees of unsaturation. The 1D NMR spectra (^{1}H and ^{13}C) displayed the signals of three methyl groups, a methoxy, an sp^3 quaternary carbon, six sp^2 methines and eight sp^2 quaternary carbons, including two carbonyl groups and two aromatic oxygenated carbons as assigned from their chemical shifts (Table 2). In the ^{1}H–^{1}H-COSY spectrum, the correlations of the two equivalent protons H-2′ and H-6′ (δ_H 7.76) with the two other equivalent ones, H-3′ and H-5′ (δ_H 7.53) which in turn were correlated to H-4′ (δ_H 7.56). This indicated the presence of a phenyl ring (**C**-ring).

Analysis of HMBC spectrum revealed the correlations of H-3 (δ_H 6.82) with C-1′ (δ_C 130.6), C-2 (δ_C 161.2), the carbonyl C-4 (δ_C 175.9) and C-10 (δ_C 116.0). Furthermore, cross-peaks of C-2 with H-2′ and H-6′ (δ_H 7.76) were observed. This observation indicated the linkages of C-2 with C-1′ and C-4 with C-10. On the other hand, the two superposed methyl groups CH_3-12 and CH_3-13 were correlated to C-9 (δ_C 176.9), C-8 (δ_C 43.0) and C-7 (δ_C 170.2). The last carbon C-7 was further correlated with the methoxy group (δ_H 3.96) and CH_3-11 (δ_H 1.98) which showed ^{3}J-HMBC correlation to the carbonyl C-5 (δ_C 183.6). These data indicated that C-8 was bonded to both CH_3-12 and CH_3-13 and C-6 linking to CH_3-11. The quaternary carbon C-10 was thus linked to both C-5 and C-9, forming the **A**-ring. The chemical shifts of C-9 and C-2 suggested that they were linked to an oxygen atom. Taking into account the molecular formula of **2**, determined that C-2 was linked to C-9 through the oxygen atom O-1. These data permitted establishing the structure of **2** as drawn in Figure 2. This unique flavone was isolated for the first time from natural source and named desmorostratone. This compound was previously described as synthetic one, forming during the synthesis of desmosdumotin B.[7]

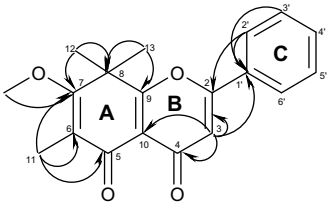


Figure 2. Keys HMBC correlations of **2**.

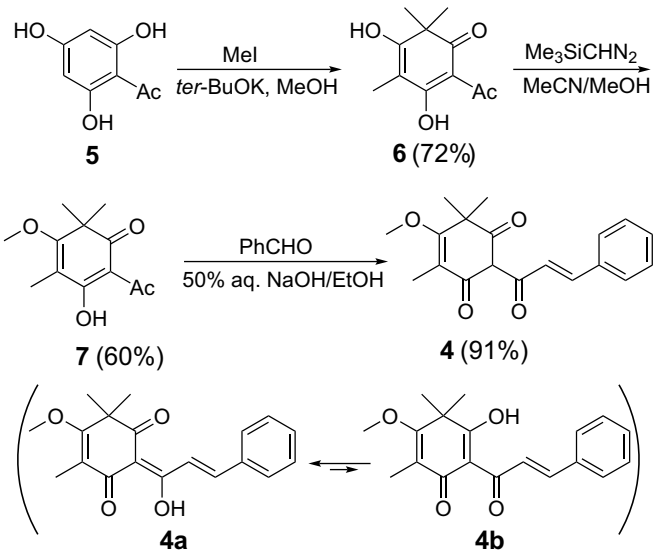
Table 1
NMR data for desmocyclopeptide (**1**) ($CDCl_3$, ^{1}H: 500.13 MHz, ^{13}C: 125.76 MHz)

Residue	δ_C	δ_H m (*J*, Hz)	Residue	δ_C	δ_H m (*J*, Hz)	Residue	δ_C	δ_H m (*J*, Hz)
Ala[1]			γ-CH_2	25.1	1.99 m	*Phe*[5]		
α-CH	49.5	4.37 m		—	2.12 m	α-CH	57.0	4.64 m
β-CH_3	15.8	1.31 d (7.5)	δ-CH_2	46.9	3.48 ddd (6.7, 6.7, 9.5)	β-CH_2	37.2	3.18 dd (7.0, 13.7)
C=O	173.5			—	3.97 ddd (7.0, 7.0, 9.5)		—	3.26 dd (9.0, 13.7)
NH		8.25 d (7.5)	C=O	172.1		1′-Phe	136.8	
Gly[2]			*Ile*[4]			2′,6′-Phe	129.4	7.22 m
α-CH_2	42.5	3.56 dd (3.2, 15.5)	α-CH	58.7	4.18 dd (8.5, 8.5)	3′,5′-Phe	128.4	7.20 m
	—	4.35 dd (5.7, 15.5)	β-CH	35.7	1.88 m	4′-Phe	126.8	7.19 m
C=O	168.5		γ-CH_2	24.9	0.97 m	C=O	172.3	
NH		7.78 dd (3.2, 5.7)		—	1.40 m	NH		7.56 d (7.5)
Pro[3]			γ′-CH_3	15.3	0.81 d (6.5)			
α-CH	62.5	4.21 dd (6.0, 8.0)	δ-CH_3	10.9	0.83 t (7.5)			
β-CH_2	29.7	1.96 m	C=O	172.3				
	—	2.29 m	NH		7.12 d (8.5)			

Table 2
NMR data for desmorostratone (**2**): ($CDCl_3$, 298 K)

Carbon	δ_C	δ_H m (*J*, Hz)	Carbon	δ_C	δ_H m (*J*, Hz)
2	161.2	—	2′	125.6	7.76 m (1.0, 7.5)
3	113.1	6.82 s	3′	129.3	7.53 m
4	175.9	—	4′	131.7	7.56 m
5	183.6	—	5′	129.3	7.53 m
6	119.2	—	6′	125.6	7.76 m (1.0, 7.5)
7	170.2	—	11	10.0	1.98 s
8	43.0	—	12	24.5	1.64 s
9	176.9	—	13	24.5	1.64 s
10	116.0	—	OMe	62.2	3.96 s
1′	130.6	—			

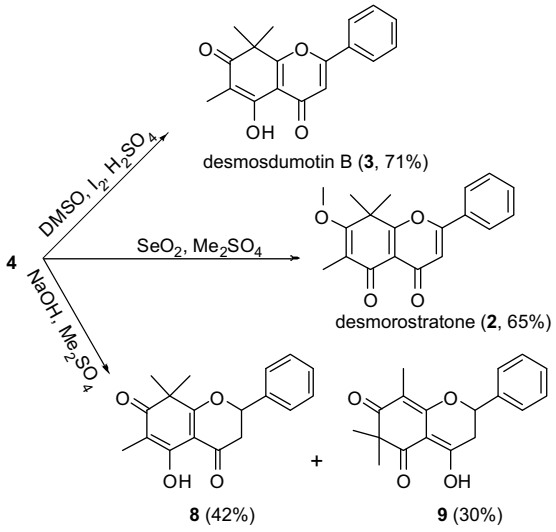
The two known compounds, desmosdumotins B (**3**)[2] and C (**4**)[3a,b] were also isolated from this plant. Their structures were established from the spectral data analysis. However, the ^{1}H NMR spectrum of **4** showed two nearly similar sets of signal with a ratio of 2.5:1. This suggested the presence of two isomers. Analysis of 2D NMR spectra of this mixture allowed assignment of NMR data for the two isomers **4a** and **4b** (Scheme 1). The two enolic forms were determined by HMBC correlations of their enolic proton with C-8 in **4a** and with C-5′ for **4b**. As mentioned above, desmosdumotin C (**4**) has been previously reported from *D. dumosus* and *C. lineatifolia* as single tautomer **4a**. In order to confirm the structures of desmorostratone (**2**) and desmosdumotin C (**4**), their total syntheses were accomplished.



Scheme 1. Synthesis of desmosmodutin C (**4**).

The syntheses of desmosdumotin B (**3**)[7] and derivatives of desmosdumotin C[8a,b] have been previously described, starting from 2,4,6-trihydroxyacetophenone. During the synthesis of **3**, desmorostratone (**2**) has been obtained as side product with low yield. Recently, a number of desmosdumotin B derivatives have been also prepared and some of them showed interesting anti tumors activity.[9] Our effort in this work was studying and optimization of the synthetic conditions to obtain desmorostratone (**2**) in high yield.

The synthesis of desmosdumotin C (**4**) was performed by using the modified method previously illustrated.[6,7] Methylation of **3** with MeI in the presence of $NaOCH_3$ gave **6** in low yield (35%). However, when using *t*-BuOK as a base, compound **6** was obtained in higher yield (72%). Exposure of **6** to a solution of Et_2O containing excess of trimethylsilanediazomethane at −18 °C yielded **7** in 60%. Condensation of **7** with benzaldehyde at room temperature provided compound **4** in good yield (91%). ^{1}H NMR spectrum of the synthetic compound was identical with that of the natural one isolating from *D. rostrata* and showed the presence of the two isomers **4a** and **4b** with a ratio of 2.5:1, respectively. This result confirmed the structure of **4** and indicated that the two enolic forms **4a** and **4b** were stable enough at room temperature and could be identified by NMR. Oxidative cyclization of **4** was firstly carried out with DMSO/I_2 at 100 °C, but the desired compound **2** was not observed. This reaction was then performed with addition of a small amount of H_2SO_4 and heated at 80 °C.[7] Under this condition, only desmosdumotin B (**3**) was obtained with 71% yield. An alternative approach was the cyclization of **2** in basic media with concurrent oxidation (Scheme 2). However, the methoxy group was cleaved off and two flavanones, **7** and **8** were obtained under this reaction condition. These results indicated that the methoxy group in the structure of **4** (or even in the structures of the cyclized products) was unstable in both acidic and basic media. This observation suggested that the oxidative cyclization of **4** should be carried out under mild conditions. Finally, desmorostratone (**2**) was obtained in good yield by treatment of **4** with SeO_2 in dimethylsulfate at 70 °C. Comparison of NMR data of the synthetic compound with the natural one revealed that they were identical.

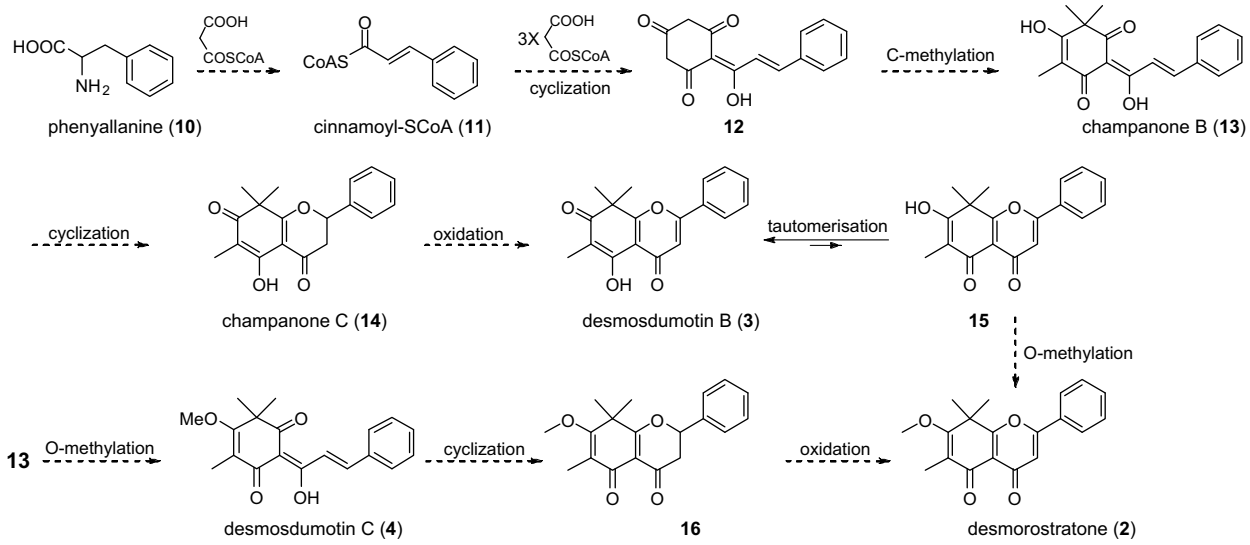


Scheme 2. Synthesis of desmorostratone (**2**).

In order to understand the formation of desmorostratone (**2**) and related compounds such as desmosdumotins B (**3**) and C (**4**) in the plants, their biosynthesis pathways were proposed (Scheme 3). As similar with the well-known biosyhthesis of chalcones, cinnamoyl-ScoA (**11**) is resulted from phenylalanine (**10**). Condensations of **11** with three malonate units following by intramolecular cyclization afford the triketone **12**. C-Methylation of **12** yields the champanone B (**13**), which has recently isolated from *C. lineatifolia*.[3b] Cyclization of **13** provides the champanone C (**14**), previously characterized from *D. dumosus* and *C. lineatifolia*.[3b,10] Oxidation of **14** yields desmosdumotin B (**3**). On the other hand, champanone (**13**) could be O-methylated affording desmosdumotin C (**4**), which should be further cyclized to give compound **16**. Finally, oxidation of **16** finishes the biosynthesis of desmorostratone (**2**). However, desmorostratone (**2**) could be also derived from desmosdumotin B (**3**) by O-methyaltion of its tautomer **15** (Scheme 3). The presence of desmosdumotin C, champanone B (**13**) and especially champanone C (**14**) in the plants supports for the hypothesis that C-methylation process is probably occurred before intramolecular cyclization of **13** and **14**.

3. Biological evaluation

In vitro cytotoxicity of the isolated compounds in human tumor cell lines (KB) was assessed as previously described.[11a,b] IC_{50} values



Scheme 3. Proposed biosynthesis pathway for desmorostratone (**2**) and related compounds.

defined, as the concentration required inhibiting cell growth by 50%, were determined after 72 h drug exposures. The results indicated that only desmosdumotin C (**4**) showed a moderate cytotoxic activity with an IC_{50} of 19.2 μM. The other compounds (**1**–**3**) presented very weak cytotoxicity on the same cell line target even when they were tested at the concentration of 25 μM.

4. Experimental section

4.1. General

Analytical TLC was performed with 0.25 mm silica gel 60 plates. Plates were developed by spraying with 5% H_2SO_4 in EtOH followed by heating on a hot plate. ^{1}H NMR spectra were recorded at 500.13 MHz and reported in ppm using tetramethylsilane (0.00 ppm) or solvent ($CDCl_3$: 7.24 ppm) as an internal standard. Data are reported as s=singlet, d=doublet, m=multiplet, br=broad; coupling constant(s) in Hz. ^{13}C NMR spectra were recorded at 125.76 MHz with chemical shifts reported in ppm (δ) in reference to the solvent peak of $CDCl_3$ (δ 77.16). The ESIMS spectra were measured by Electrospray MS in the positive mode.

4.2. Plant material and extraction

The plant *D. rostrata* P.T. Li was collected in Hatinh-Vietnam in March 2004 and a specimen (VN 1273) was deposited at the Institute of Ecology and Biological Resources—VAST—Vietnam. Dried and ground stem bark (1.0 kg) of *D. rostrata* were extracted with MeOH at room temperature for three times (3×4 L). The solvent was removed under diminished pressure. The crude MeOH extract was suspended in water and extracted successively with *n*-hexane and then with CH_2Cl_2. The CH_2Cl_2 extract solution was concentrated under diminished pressure and the residue (5.3 g) was purified by repeated open column chromatography over silica gel, eluted with a mixture of CH_2Cl_2/MeOH to afford desmocyclopeptide (**1**, 25 mg), desmorostratone (**2**, 16 mg), desmosdumotin B (**3**, 125 mg) and desmosdumotin C (**4**, 86 mg).

4.3. Desmocyclopeptide (1): $C_{25}H_{35}N_5O_5$

White solid (Et_2O/EtOH), mp 261–262 °C; R_f 0.37 (CH_2Cl_2/MeOH: 92/8); $[\alpha]_D^{20}$ −126.7 (*c* 2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 241.6 (2.98), 283.0 (2.45); ν_{max} (KBr) 3542, 3477, 3341, 2969, 2876, 1651, 1531, 1437 cm^{-1}; HRMS (ESI): $[M+H]^+$, found 486.2727. $C_{25}H_{36}N_5O_5$ requires 486.2716; ESI-MS/MS on $[M+H]^+$ ion, *m*/*z*: 486, 458, 339, 311, 268, 240, 211, 183, 114, 86; NMR data see Table 1.

4.4. Desmorostratone (2): $C_{19}H_{18}O_4$

Yellow solid (*n*-hexane/EtOAc), mp 199–201 °C; R_f 0.29 (*n*-hexane/EtOAc: 9:1); UV (MeOH) λ_{max} (log ε) 213.4 (2.44), 253.0 (2.85), 274.9 (2.89); ν_{max} (KBr) 1675, 1632, 1451, 1405, 1123 cm^{-1}; HRMS (ESI): $[M+H]^+$, found 311.1279. $C_{19}H_{19}O_4$ requires 311.1283; NMR data see Table 2.

4.5. Desmosdumotin B (3): $C_{18}H_{16}O_4$

Yellow solid (*n*-hexane/EtOAc), mp 215–216 °C; R_f 0.75 (CH_2Cl_2/MeOH: 98:2); ν_{max} (KBr) 3435, 2934, 1685, 1635, 1602, 1555, 1446, 1427, 1294, 1161 cm^{-1}; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H 13.05 (1H, s, chelated-OH), 7.81 (2H, m, *J* 7.0 Hz, H-2′ and H-6′), 7.61 (1H, m, *J* 7.0 Hz, H-4′), 7.57 (2H, m, H-3′ and H-5′), 6.89 (1H, s, H-3), 1.88 (3H, s, Me-11), 1.59 (6H, s, Me-12 and Me-13); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 196.2 (C-7), 180.8 (C-4), 174.1 (C-9), 164.3 (C-2), 163.5 (C-5), 132.6 (C-4′), 130.0 (C-1′), 129.5 (C-3′ and C-5′), 126.0 (C2′ and C-6′), 110.5 (C-10), 110.2 (C-3), 108.6 (C-6), 47.3 (C-5), 25.3 (C-12 and C-13), 7.0 (C-11); HRMS (ESI): $[M+H]^+$, found 297.1121. $C_{18}H_{17}O_4$ requires 297.1127.

4.6. Desmosdumotin C (4): $C_{19}H_{20}O_4$

Yellow solid (*n*-hexane/EtOAc), mp 93–94 °C; R_f 0.52 (*n*-hexane/EtOAc: 9:1); ν_{max} (KBr) 3434, 2934, 1664, 1616, 1573, 1519, 1419, 1202, 1116 cm^{-1}; **4a**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H 19.14 (1H, s, chelated-OH), 8.26 (1H, d, *J* 16.0 Hz, H-8), 7.86 (1H, d, *J* 16.0 Hz, H-7), 7.59 (1H, m, H-4), 7.32 (4H, m, H-2, H-3, H-5 and H-6), 3.88 (3H, s, OMe), 1.93 (3H, s, Me-7′), 1.31 (6H, s, Me-8′ and Me-9′); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 198.0 (C-6′), 192.4 (C-2′), 187.2 (C-9), 176.6 (C-4′), 144.8 (C-7), 135.3 (C-1), 130.6 (C-3 and C-5), 129.0 (C-4), 128.9 (C-2 and C-6), 123.3 (C-8), 113.6 (C-3′), 106.6 (C-1′), 62.1 (OMe), 50.4 (C-5′), 24.3 (Me-8′ and Me-9′), 9.8 (Me-7′); **4b**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H 18.72 (1H, s, chelated-OH), 8.47 (1H, d, *J* 16.0 Hz, H-8), 7.88 (1H, d, *J* 16.0 Hz, H-7), 7.61 (1H, m, H-4), 7.32 (4H, m, H-2, H-3, H-5, H-6), 3.82 (3H, s, OMe), 1.88 (3H, s, CH_3-7′), 1.40 (6H, s, CH_3-8′, CH_3-9′); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 201.6 (C-6′), 189.5 (C-9), 186.2 (C-2′), 171.0 (C-4′), 145.4 (C-7), 135.2 (C-1), 130.7 (C-3 and C-5), 129.0 (C-2 and C-6), 123.7 (C-8), 118.5 (C-3′), 108.4 (C-

1′), 61.9 (OMe), 46.1 (C-5′), 24.4 (Me-8′ and Me-9′), 10.2 (Me-7′); HRMS (ESI): $[M+H]^+$, found 313.1446. $C_{19}H_{21}O_4$ requires 313.1440.

*4.6.1. Preparation of **6***

A solution of 2,4,6-trihydroxyacetophenone (**5**, 982 mg, 5.8 mmol) and potassium *tert*-butoxide (2.4 g, 21.4 mmol) in anhydrous MeOH (70 mL) containing methyliodide (1.2 mL, 19.2 mmol) was heated to reflux for 7 h. The reaction mixture was cooled to 0 °C and acidified with 1 N HCl aqueous solution, then extracted three times with EtOAc (3×30 mL). The combined organic layers were washed with water (3×50 mL), dried over Na_2SO_4 and concentrated under diminished pressure. The residue was chromatographed on silica gel colunm, eluted with *n*-hexane/EtOAc (9:1) to provide **6** (876 mg, 72%). Yellow solid, mp 164–165 °C (*n*-hexane/EtOAc); R_f 0.45 (CH_2Cl_2/MeOH: 97:3); ν_{max} (KBr) 3123, 1665, 1596, 1532, 1378, 1338, 1278 cm^{-1}; ^1H NMR (500.13 MHz, DMSO-d_6): δ_H 18.93 (1H, br s, chelated-OH), 2.47 (3H, s, Me–Ac), 1.78 (3H, s, Me), 1.28 (6H, s, 2×Me); ^{13}C NMR (125.76 MHz, DMSO-d_6): δ_C 199.8, 196.5, 189.1, 175.1, 113.8, 106.1, 47.6, 24.7, 7.7; HRMS (ESI): $[M+H]^+$, found 211.0975. $C_{11}H_{15}O_4$ requires 211.0970.

*4.6.2. Preparation of **7***

To a solution of **6** (545 mg, 2.6 mmol) in a mixture of CH_3CN/MeOH (2:1; 7.5 mL), was added slowly a solution of $(CH_3)_3SiCHN_2$ in diethyl ether (5 mL, 10 mmol) at −18 °C and the resulting mixture was stirred for 3 h. Acetic acid was then added to destroy the excess of $(CH_3)_3SiCHN_2$. The mixture was concentrated under diminished pressure and the residue was purified by silica gel column chromatography, eluted with *n*-hexane/EtOAc (9:1) to afford **7** (352 mg, 60%) as yellow oil. R_f 0.63 (CH_2Cl_2/MeOH: 97:3); ν_{max} (KBr) 3112, 1661, 1602, 1526, 1374, 1331, 1279 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$) (mixture of two tautomers): δ_H 18.91 (s) and 18.13 (s) (2:1, chelated-OH), 3.94 (s) and 3.87 (s) (2:1, OMe), 2.70 (s) and 2.61 (s) (1:2, C(O)Me), 1.97 (s) and 1.91 (s) (2:1, Me), 1.44 (s) and 1.33 (s) (2:1, 2×Me); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 199.6, 199.1, 192.5, 192.1, 189.2, 189.5, 173.1, 172.4, 118.1, 117.3, 107.6, 107.2, 61.9, 61.7, 46.5, 46.3, 24.6, 24.5, 10.7, 10.2; HRMS (ESI): $[M+H]^+$, found 225.1122. $C_{12}H_{17}O_4$ requires 225.1127.

*4.6.3. Preparation of desmosdumotin C (**4**)*

A solution of **7** (330 mg, 1.47 mmol) in EtOH (15 mL) and 50% KOH in water (10 mL), was treated with benzaldehyde (1.5 mL, 1.48 mmol). The resulting solution was stirred at room temperature for 24 h. The reaction mixture was poured into 1 N HCl aqueous solution, and then extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4 and concentrated under diminished pressure. The residue was chromatographed on silica gel, eluted with *n*-hexane/EtOAc (9:1, v/v) to obtain 4 (417 mg, 91%) as tautomeric mixtures **4a** and **4b** with a ratio of 2.5:1, respectively. Yellow solid (*n*-hexane/EtOAc), mp 92–96 °C; NMR-spectra are identical with those of the natural compound.

*4.6.4. Preparation of **8** and **9***

A solution of Me_2SO_4 (2 mL), containing **4** (20 mg, 0.06 mmol) was treated with NaOH (2.56 mg, 0.06 mmol) at room temperature for 12 h. The reaction solution was then neutralized with 1 N HCl aqueous solution to pH ∼7 and then extracted with EtOAc (3×10 mL). The solvent was removed under diminished pressure and then the residue was purified by preparative TLC (30% EtOAc/*n*-hexane) to provide **8** (8 mg, 42% yield) and **9** (5.7 mg, 30% yield). **8**: R_f 0.46 (CH_2Cl_2/MeOH: 97:3); ^1H NMR (500.13 MHz, $CDCl_3$): δ_H 11.61 (1H, s, chelated-OH), 7.48–7.40 (5H, m, phenyl ring), 5.58 (1H, dd, *J* 3.5, 13.5 Hz, H-2), 3.10 (1H, dd, *J* 13.5, 17.0 Hz, H-3_{ax}), 2.88 (1H, dd, *J* 3.5, 17.0 Hz, H-3_{eq}), 1.81 (3H, s, Me), 1.45 (3H, s, Me), 1.41 (3H, s, Me); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 196.6 (C-7), 194.5 (C-4), 186.0 (C-9), 164.0 (C-5), 136.3 (C-1′), 129.6 (C-4′), 129.1 (C-3′ and C-5′), 126.0 (C-2′ and C-6′), 105.7 (C-6), 103.2 (C-10), 81.2 (C-2), 48.5 (C-8), 41.7 (C-3), 24.8 (Me), 24.7 (Me), 6.7 (Me); HRMS (ESI): $[M+H]^+$, found 299.1279. $C_{18}H_{19}O_4$ requires 299.1283; **9**: R_f 0.56 (CH_2Cl_2/MeOH: 97:3); ^1H NMR (500.13 MHz, $CDCl_3$): δ_H 15.80 (s, chelated-OH, 1H); 7.47–7.39 (5H, m, phenyl ring), 5.32 (1H, dd, *J* 3.5, 11.0 Hz, H-2), 3.03 (1H, dd, *J* 11.0, 18.0 Hz, H-3_{ax}), 2.92 (1H, dd, *J* 3.5, 18.0 Hz, H-3_{eq}), 1.86 (3H, s, Me), 1.42 (3H, s, Me), 1.40 (3H, s, Me); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ_C 201.8 (C-5), 198.1 (C-7), 182.9 (C-4), 161.3 (C-9), 138.1 (C-1′), 129.0 (C-3′ and C-5′), 128.9 (C-4′), 125.8 (C-2′ and C-6′), 107.4 (C-8), 101.5 (C-10), 76.1 (C-2), 52.4 (C-6), 38.3 (C-3), 25.5 (Me), 23.1 (Me), 7.9 (Me); HRMS (ESI): $[M+H]^+$, found 299.1286. $C_{18}H_{19}O_4$ requires 299.1283.

*4.6.5. Preparation of desmorostratone (**2**)*

A solution of **4** (100 mg, 0.32 mmol) in Me_2SO_4 (10 mL) was stirred at 70 °C for 1 h, and then SeO_2 was added and continuously stirred for additional 3 h. The reaction mixture was extracted with Et_2O (3×15 mL). The combined organic extracts were washed with water, dried over Na_2SO_4 and concentrated under diminished pressure. The residue was chromatographed on silica gel column, eluted with CH_2Cl_2/MeOH (20:1, v/v), recrystallization from *n*-hexane/EtOAc gave desmorostratone (**2**) (66 mg, 65%). Yellow solid (*n*-hexane/EtOAc), mp 197–199 °C; NMR-spectra are identical with those of the natural compound.

4.6.6. Peptide hydrolysis

Desmocyclopeptide (**1**) sample (3.0 mg) was dissolved in 6 N HCl (1 mL) and heated at 110 °C for 24 h. The solvent was removed under reduced pressure and the resulting material was subjected to further derivatisation.

4.6.7. HPLC analysis of Marfey's (FDAA) derivatives

The hydrolysate mixture (3.5 mg) or the amino acid standards (0.5 μg) was dissolved in 0.1 mL of water and treated with 0.2 mL of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) in acetone and 0.04 mL of 1.0 M sodium bicarbonate. The vials were heated at 50 °C for 90 min and the contents after cooling at room temperature, were neutralised with 1 N HCl. After degassing an aliquot of the FDAA derivative was diluted with MeOH and chromatographed on a RP C-18 column (250×4.6 mm) by means of a linear gradient of acetonitrile and water containing 0.05% trifluoroacetic acid from 20:80 to 50:50 in 20 min and then isocratic. The flow rate was 1 mL/min and the absorbance detection was at 340 nm. The chromatogram was compared with those of standards treated in the same conditions.

Amino acids in desmocyclopeptide (**1**): Gly (13.8 min), L-Ala (14.9 min), L-Pro (15.5 min), L-Ile (21.0 min), L-Phe (21.5 min). T_R of standards: L-Ala (14.9 min), D-Ala (16.1 min), L-Pro (15.5 min), L-Ile (21.0 min), L-Phe (21.5 min), D-Phe (23.9 min).

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Supplementary data

^1H and ^{13}C NMR spectra of the isolated compounds. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.06.017.

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