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A New Red Pigment from Chinese Dragon's Blood, the Red Resin of *Dracaena cochinchinensis*

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Dragon's blood is a non-specific name for deep red resinous exudations from plants. It has been used as a famous medicinal herb, but also as a colourant in works of art since ancient times by many cultures.[1] The deep resin is from quite different plant taxa endemic to various regions around the globe, *i.e*. *Dracaena* (Agavaceae) originally in Middle-east Asia and North Africa, *Daemonorops* (Palmae) from Southeast Asian, Malaysia and Indonesia, *Croton* (Euphorbiaceae) in south American Amazon valley region, and *Pterocarpus* (Leguminosa) in India.[2] In China, the red resin from *Dracaena* was firstly imported through the silk road in Sui and Tang dynasties and used widely as an important traditional Chinese medicinal herb.[3,4] Until 1970s, the red resin of *D. cochinchinensis* S. C. Chen used originally by the Dai people living in the south part of Yunnan province, China, for treating hurt and stopping blood, has been found and used to be the substitute of the traditionally imported dragon's blood, called Long-Xue-Jie (Chinese dragon's blood).[3]

To date, a series of publications reported the chemistry and therapeutic uses of various dragon's blood and their original plants.[2,5-10] Several pigments, such as dracorhodin (**2**) and dracorubin reported from *Daemonorops* resin, elucidated the reason that dragon's blood takes on red color.[11-14] Moreover, HPLC and GC/MS methods were developed to identify pigments and discriminate dragon's blood from different individual species.[1,15-18] As our continuing studies on Chinese dragon's blood and its original plants, some steroids were mainly identified from the fresh stems of the original plant of *D. cochinchinensis*,[19-23] while phenolic compounds were found to be the main constituents in the red resins of the plant.[24-26] However, the pigments of the red resins from *D. cochinchinensis* were pressing for researching. This paper describes the isolation and determination of a novel red pigment **1** from Chinese dragon's blood, the red resin of *D. cochinchinensis*. It is the first time to isolate and determine the pigments from *Dracaena* resins.

Compound **1** was a deep red amorphous powder and possessed a molecular formula $C_{32}H_{28}O_7$, as deduced from the negative ion HRESIMS (*m/z* 523.1747 $[M-H]^-$) and the ^{13}C NMR (Table 1). Thirty-two carbon resonances were well resolved in the ^{13}C NMR spectrum (Table 1) and further classified by DEPT and HMQC experiments as one carbonyl (δ 185.3), 12 quaternary aromatic carbons (δ 116-163), one aliphatic (δ 39.1) and 14 aromatic methines, two aliphatic methylenes (δ 32.9 and 30.4) and two aromatic linked methoxyls (δ 56.5 and 55.4). The aromatic region of the ^1H NMR spectrum of **1** displayed two sets of AA'XX' coupled signals at δ 7.59, 6.85 (each 2H, d, *J* = 8.8 Hz) and δ 7.23, 6.67 (each 2H, d, *J* = 8.4 Hz) arising from two 1,4-disub-

Table 1. ^{13}C NMR and ^1H NMR data of compound **1** (CD_3OD)

Positions	δ_H (mult *J* in Hz)	δ_C (mult)
H-α	2.53 (2H, m)	32.9 (t)
H-β	2.43 (2H, m)	30.4 (t)
CH	2.43 (1H, m)	39.1 (d)
2	-	163.9 (s)
3	6.92 (1H, d, *J* = 7.8 Hz)	102.6 (d)
4	8.01 (1H, d, *J* = 7.8 Hz)	138.0 (d)
4a	-	116.3 (s)
5	-	157.5 (s)
6	-	122.7 (s)
7	-	185.3 (s)
8	6.17 (1H, s)	105.3 (d)
8a	-	159.5 (s)
1'	-	123.0 (s)
2',6'	7.59 (2H, d, *J* = 8.8 Hz)	129.9 (d)
3',5'	6.85 (2H, d, *J* = 8.8 Hz)	117.2 (d)
4'	-	163.1 (s)
1"	-	122.7 (s)
2"	-	158.6 (s)
3"	6.19 (1H, d, *J* = 2.3 Hz)	99.5 (d)
4"		156.8 (s)
5"	6.13 (1H, dd, *J* = 8.0, 2.3 Hz)	107.4 (d)
6"	6.73 (1H, d, *J* = 8.0 Hz)	131.9 (d)
1"'	-	136.9 (s)
2"',6"'	7.23 (2H, d, *J* = 8.4 Hz)	129.6 (d)
3"',5"'	6.67 (2H, d, *J* = 8.4 Hz)	115.8 (d)
4"'	-	156.3 (s)
OCH_3	3.44 (3H, s)	55.4 (q)
OCH_3	3.82 (3H, s)	56.5 (q)

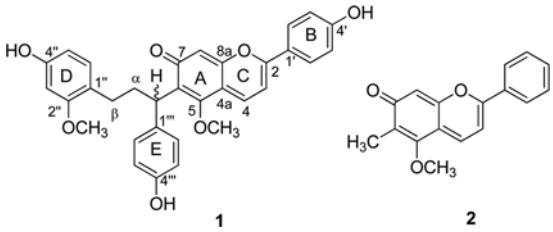

Figure 1. Pigments **1** and **2** from *Dracanea* (**1**) and *Daemonorops* (**2**) resins.

stituted aromatic rings. In addition, one ABX coupled signals at δ 6.73 (d, J = 8.0 Hz), 6.19 (d, J = 2.3 Hz), and 6.13 (dd, J = 8.0, 2.3 Hz) ascribable to one 1,2,4-tri-substituted aromatic ring, together with two AX coupled signals at δ 8.01, 6.92 (each 1H, d, J = 7.8 Hz) and one singlet aromatic proton at δ 6.17 (s).

Detailed analysis of the ^{1}H-^{1}H COSY, HMQC, and HMBC spectra (Fig. 2) further confirmed that **1** was a biflavonoid composing of a deoxotetrahydrochalcone and a 2-phenyl-chromen-7-one units. The ^{1}H-^{1}H COSY correlations of two aliphatic methylenes ($CH_2(\alpha)$-$CH_2(\beta)$) were observed. One aliphatic methylene (CH_2, β) was elucidated to be linked with the 1,3,4-tetrasubstituted aromatic ring (D ring), by the HMBC correlations of the methylene proton (δ 2.43, CH_2, β) to δ 122.7 (C-1")/158.6 (C-2")/131.9 (C-6") from D ring and the aromatic proton at δ 6.73 (H-6") to δ 30.4 (CH_2, β)/158.6 (C-2")/156.8 (C-4"). The HMBC cross peaks of H-3" (δ 6.19) with C-1"/C-2"/C-4"/C-5" (δ 107.4), and H-5" (δ 6.13) with C-3" (δ 99.5)/C-1"/C-4", as well as the methoxyl protons at δ 3.82 (s) with C-2" confirmed the 2"-methoxy-4"-hydroxy substitution of D-ring. In addition, the HMBC correlations of the aromatic protons at δ 7.23 (d, J = 8.4 Hz, H-2"' and 6"') from E ring with the methine carbon at δ 39.1 (CH) were observed. The above data indicated a deoxo-tetrahydrochalcone unit existing in compound **1**, which was similar to that of (2*R*)-8-methylsocotrin-4'-ol, a flavonoid derivative from Chinese dragon's blood.[8] Moreover, in the HMBC spectrum (Fig. 2), the correlations of δ 7.59 (d, J = 8.8 Hz, H-2' and 6') from B ring with C-2 (δ 163.9)/C-4' (δ 163.1) were observed. The AX coupled aromatic protons at δ 6.92 (H-3) and δ 8.01 (H-4) assignable to C ring were correlated to C-2/C-4a (δ 116.3)/C-1' (δ 123.0), and C-2/C-4a/C-8a (δ 159.5)/C-5 (δ 157.5), respectively. These correlations together with the HMBC correlations of the methoxy proton at δ 3.44 (s) with C-5 from A ring and the singlet aromatic proton at δ 6.17 (1H, s, H-8) with C-4a/C-6 (δ 122.7) suggested the existence of a 2-phenyl-5-methoxy-chromen-7one unit. This was similar to dracorhodin (2-phenyl-5-methoxy-6-methyl-chromen-7one, **2**), one of the known pigment reported from *Daemonorops* resin.[27] The connection of the deoxotetrahydrochalcone unit to C-6 position of the chromen unit was supported by the key HMBC correlations from methylene proton at δ 2.53 (CH_2, α) with C-6 from A ring and the methine proton (CH) with C-5, C-6 and methylene carbon (CH_2, δ). Therefore, compound **1** was determined as shown in Figure 1 and named as dracaenin A.

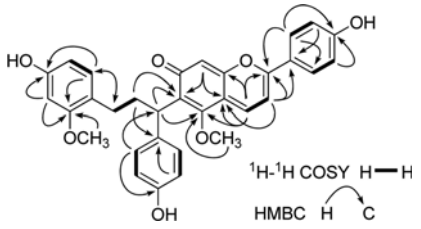

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compound **1**.

In conclusion, the novel oxydic biflavonoids **1** composing of a deoxotetrahydrochalcone unit and a flavane with quinoid unit, was identified from the red resin of *D. cochinchinesis*. Although a series of phenolic compounds were reported from dragon's blood and its original plants, no pigment was isolated from *Dracaena* resins. This paper present the first determination of the red pigments from the red *Dracaena* resins. The compound showed deep red color, which should elucidate the reason for the red color of Chinese dragon's blood.

Experimental

General Procedures. Optical rotations were measured with an HORIBA SEPA-300 high-sensitive polarimeter. NMR spectra were run on Bruker AV-400 (for ^{1}H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR) instruments with TMS as internal standard; ESIMS and HRESIMS spectra were recorded on a VG Auto Spec-3000 spectrometer. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. Silica gel (200-300 mesh and 10-40 μm), RP-18 (40-63 μm) and Sephadex LH-20 were used for column chromatography. Precoated silica gel plates (Merck) were used for TLC. Detection was done by spraying plates with 10% sulfuric acid-EtOH, followed by heating.

Plant Material. The red resin of *D. cochinchinensis* was purchased from Weihe Pharmaceutical Company (Yuxi, Yunnan, P. R. China). A sample was deposited in our laboratory. Identification of the extract was supported by an HPLC comparison with an authentic sample, which was Long-Xue-Jie (Chinese dragon's blood, No. 020624) provided by Xishuangbanna Botanical Garden, Chinese Academy of Sciences. A voucher specimen of *D. cochinchinensis* (KUN 0238050) is deposited in State Key Laboratory of Phytochemistry and Plant Resources in west China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The red resin (3.0 kg) of *D. cochinchinensis* was extracted with 90% aqueous ethanol under reflux three times, each time 2 h. After removal of the solvent, "Long-Xue-Jie" (Chinese dragon's blood, 1.2 kg) was obtained. Chinese dragon's blood (1.0 kg) was further extracted with $CHCl_3$ and EtOAc, successively. The EtOAc extract (700 g) was subjected to a silica gel column, eluting with $CHCl_3$, $CHCl_3$/MeOH (20:1, 10:1, 10:2), and MeOH (each 2 L), successively, to give six fractions. Fraction 5 (30.0 g) containing mainly pigments, was subjected to repeated

column chromatography on silica gel ($CHCl_3$/MeOH, 20:1-4:1), RP-18 (MeOH/H_2O, 6:4-1:0) and Sephadex LH-20 (MeOH/H_2O, 3:7-1:0) to yield one claret composition, dracaenin A (**1**) (23 mg).

Dracaenin A (1): $[\alpha]_D^{20}$ −93.3 (*c* 0.01, MeOH); deep red amorphous powder; UV (MeOH) λ_{max} nm (log ε): 204 (1.26), 281 (0.23), 341 (0.09), 387 (0.08), 529 (0.12); ^1H and ^{13}C NMR data, see Table 1; ESIMS (negative ion mode): *m/z* 523 $[M-H]^-$; negative ion HRESIMS: *m/z* 523.1747 $[M-H]^-$ (calcd for $C_{32}H_{27}O_7$, 523.1756).

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References

1. Baumer, U.; Dietemann, P. *Anal. Bioanal. Chem.* **2010**, *397*, 1363.
2. Gupta, D.; Bleakley, B.; Gupta, R. K. *J. Ethnopharmacol.* **2008**, *115*, 361.
3. Cai, X. T.; Xu, Z. F. *Acta Bot. Yunnan.* **1979**, *1*, 1.
4. Zheng, Q. A.; Chen, J. T.; Zhang, Y. J.; Yang, C. R. *Acta Bot. Yunnan.* **2003**, Suppl. XIV, 102.
5. Camarda, L.; Merlini, L.; Nasini, G. *Heterocycles* **1983**, *20*, 39.
6. Gonzalez, A. G.; Leon, F.; Sanchez-Pinto, L.; Padron, J. I.; Bermejo, J. *J. Nat. Prod.* **2000**, *63*, 1297.
7. Gonzalez, A. G.; Leon, F.; Hernandez, J. C.; Padron, J. I.; Sanchez-Pinto, L.; Bermejo Barrera, J. *Biochem. Syst. Ecol.* **2004**, *32*, 179.
8. Zhu, Y. D.; Zhang, P.; Yu, H. P.; Li, J.; Wang, M. W.; Zhao, W. M. *J. Nat. Prod.* **2007**, *70*, 1570.
9. Yong, K. L.; Lv, J. C.; Zhang, T. B.; Xu, L. R.; Chen, X. *Nat. Prod. Res.* **2008**, *22*, 1624.
10. Wen, D. X. *Chinese Traditional and Herbal Drugs* **2001**, *32*, 1053.
11. Brockmann, H.; Haase, R.; Freiensehner, E. Ber. Dtsch. *Chem. Ges. B* **1944**, *77B*, 279.
12. Collins, D. A.; Haworth, F.; Isarasena, K.; Robertson, A. *J. Chem. Soc.* **1950**, 1876.
13. Olaniyi, A. A.; Powell, J. W.; Whalley, W. B. *J. Chem. Soc.* **1973**, *2*, 179.
14. Robertson, A.; Whalley, W. B.; Yates, J. *J. Chem. Soc.* **1950**, 3117.
15. Li, Y. C.; Wang, Y. Y.; Song, H. M.; Li, Z. G. *Journal of Nanjing University of Traditional Chinese Medicine* **2011**, *27*, 396.
16. Lu, J.; Liu, Y.; Wang, B. Q. *China Journal of Chinese Materia Medica* **1991**, *16*, 615.
17. Wang, H. P.; Ning, L. F.; Zhang, S. S.; Feng, D.; Zhou, Q. F.; Yang, Q. L. *J. Toxicol.* **2010**, *24*, 234.
18. Sousa, M. M.; Melo, M. J.; Parola, A. J.; Seixas de Melo, J. S.; Catarino, F.; Pina, F.; Cook, F. E. M.; Simmonds, M. S. J.; Lopes, J. A. *J. Chromatogr. A* **2008**, *1209*, 153.
19. Zheng, Q. A.; Li, H. Z.; Zhang, Y. J.; Yang, C. R. *Steroids* **2006**, *71*, 160.
20. Zheng, Q. A.; Yang, C. R. *Chin. Chem. Let.* **2003**, *14*, 1261.
21. Zheng, Q. A.; Yang, C. R. *J. Asian Nat. Prod. Res.* **2003**, *5*, 291.
22. Zheng, Q. A.; Zhang, Y. J.; Li, H. Z.; Yang, C. R. *Steroids* **2004**, *69*, 111.
23. Zheng, Q. A.; Zhang, Y. J.; Yang, C. R. *J. Asian Nat. Prod. Res.* **2006**, *8*, 571.
24. Zheng, Q. A.; Li, H. Z.; Zhang, Y. J.; Yang, C. R. *Helv. Chim. Acta* **2004**, *87*, 1167.
25. Zhou, Z. H.; Wang, J. L.; Yang, C. R. *Acta Pharmaceutica Sinica* **2001**, *36*, 200.
26. Zhou, Z. H.; Wang, J. L.; Yang, C. R. *Chinese Traditional and Herbal Drugs* **2001**, *32*, 484.
27. Shen, C. C.; Tsai, S. Y.; Wei, S. L.; Wang, S. T.; Shieh, B. J.; Chen, C. C. *Nat. Prod. Res.* **2011**, *21*, 377.