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Article

Cochinchin from *Dracaena cochinchinensis*

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A new compound, Cochinchin (**1**), together with 7,4'-dihydroxyflavone (**2**), 7-hydroxy-4'-methoxyflavane (**3**), 7-hydroxy-3-(4-hydroxybenzyl)chroman (**4**), 4'-hydroxy-2,4-dimethoxydihydrochalcone (**5**) and 4'-hydroxy-2,4,6-trimethoxydihydrochalcone (**6**) was isolated from the resin (trivial name, "dragon's blood") of *Dracaena cochinchinensis* (Lour.) S. C. Chen. The structure of **1** was elucidated on the basis of spectroscopic data as (2,3-*trans*)-6-allyl-2-(3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)-2,3-dihydrobenzo[1,4]dioxin which is a natural product possessing a new framework.

Keywords *Dracaena cochinchinensis*, Cochinchin, isolation, phenol

Introduction

Phytochemical studies on *D. cochinchinensis* (Lour.) S. C. Chen. (*Liliacaece*) have previously led to the isolation of a variety of flavonoids, steroidal saponins and tritepenoids.[1] Biologically the red resin of "Dragon's blood" originated from *D. cochinchinensis* has been shown to be antiinflammatory, analgesic, blood-activating stasis-dispelling, *etc*.[1] Investigation on the EtOAc extract of the red resin has led to the isolation of cochinchin bearing a rarely typed 2,3-dihydrobenzo-[1,4]dioxin skeleton (**1**) (Figure 1), along with 7,4'-dihydroxyflavone (**2**), 7-hydroxy-4'-methoxyflavane (**3**), 7-hydroxy-3-(4-hydroxybenzyl)chroman (**4**), 4'-hydroxy-2,4-dimethoxydihydrochalcone (**5**), and 4'-hydroxy-2,4,6-trimethoxydihydrochalcone (**6**). This article deals mainly with the structural determination of the novel compound **1**.

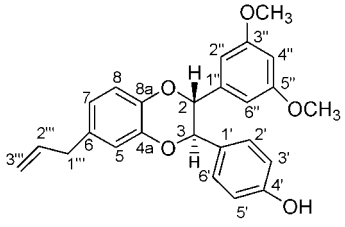

Figure 1 Figured diagram of (2,3-*trans*)-6-allyl-2-(3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)-2,3-dihydrobenzo[1,4]dioxin.

Results and discussion

Compound **1** was obtained as brown oil. Its molecular formula was deduced as $C_{25}H_{24}O_5$ by its HRFABMS where the molecular ion of **1** was displayed at *m/z* 404.16152 (calcd for $C_{25}H_{24}O_5$: 404.16181, $\Omega=14$). The IR absorption bands at 3390, 1597, 1515, 1504 and 1462 cm^{-1} suggested the presence of hydroxyl(s), double bond(s) and/or benzene ring(s). The ^{13}C NMR spectrum of **1** edited by the DEPT pulse sequences gave a total of eight quaternary, thirteen methine, two methylene and two methoxy carbons (Table 1) demonstrating the presence of two chemically equivalent arylic methoxy groups (δ_C 55.7), two oxygenated methines (δ_C 80.6 and 81.2) and three benzene nuclei (δ_C 160.9, 160.9, 156.1, 144.0, 142.3, 138.9, 134.0, 129.4, 129.4, 129.1, 122.0, 117.5, 117.4, 115.5, 115.5, 105.9, 105.9, and 101.4) in addition to a vinyl methyl moiety (δ_C 116.1, 137.9 and 39.9). The ^{13}C NMR data of **1** suggested that it was most probably an adduct of stilbenoid and benzylpropene derivative. This assumption was subsequently confirmed by the ^1H NMR spectrum of **1** as well as ^1H-^1H COSY, HMQC, ROESY, and HMBC experiments allowing the assignment of all proton and carbon signals (Table 1). In addition to a six-proton singlet at δ_H 3.69 arising from two methoxy groups, the ^1H NMR spectrum of **1** indicated that the three benzene nuclei were 1,4-di- [δ_H 7.01 and 6.74 (d, $J=8.4$ Hz, 2H each)], 1,3,5-tri- [δ_H 6.36 (1H, brs) and 6.23 (2H, brs)], and 1,2,4-trisubstituted [δ_H 7.00 (d, $J=8.2$ Hz, 1H), 6.78 (d, $J=8.1$ Hz, 1H) and 6.92 (brs, 1H)]. Furthermore, two mutually coupled oxygenated methines appeared downfield at δ_H 4.81 (d, $J=15$ Hz, 1H), and 4.83 (d, $J=15$ Hz, 1H) indicating that they connected two of the three phenyl rings to form a stilbenoid moiety, and the benzylpropene residue was composed of the third benzene ring and an allyl group giving a set of signals at

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δ_H 5.98 (m, 1H), 5.09 (dd, J =18, 1.3 Hz, 1H), 5.07 (d, J =10 Hz, 1H), and 3.36 (d, J =6.6 Hz, 2H).

The carbon skeleton and substitution pattern of **1** was assigned using HMBC experiment. The 3″,5″-dimethoxy groups were indicated by HMBC correlation of the six-proton singlet at δ_H 3.69 with C-3″,5″ at δ_C 160.9, both correlating further to H-2″,6″ (δ_H 6.23). The correlations of H-2 (δ_H 4.81) with C-3 (δ_C 80.6), C-1″ (δ_C 138.9), C-2″,6″ (δ_C 105.9) established the connectivity of the aromatic ring to the oxygenated methine. The HMBC coupling from H-2′,6′ (δ_H 7.01) to C-3 (δ_C 80.6), C-4′ (δ_C 156.1), C-5′,3′ (δ_C 115.5), C-1′ (δ_C 129.1), H-3′, 5′ (δ_H 6.74) to C-1′ (δ_C 129.1), C-2′, 6′ (δ_C 129.4), C-4′ (δ_C 156.1), and H-3 (δ_H 4.83) to C-2 (δ_C 81.2), C-1′ (δ_C 129.1), C-2′, 6′ (δ_C 129.4) indicated that the aromtic ring was a 4-hydroxylphenyl group. The HMBC coupling from H-8 (δ_H 7.00) to C-6 (δ_C 134.0), C-7 (δ_C 122.0), C-4a (δ_C 144.0), C-8a (δ_C 142.3), the coupling from H-7 (δ_H 6.78) to C-5 (δ_C 117.4), C-6 (δ_C 134.0), C-8 (δ_C 117.5), C-8a (δ_C 142.3), C-1‴ (δ_C 39.9), and the coupling from H-5 (δ_H 6.92) to C-4a (δ_C 144.0), C-7 (δ_C 122.0), C-8a (δ_C 142.3), C-1‴ (δ_C 39.9) indicated the presence of a 6-allyl-phenyl. Then, all the above evidences proved the presence of a 6-allyl-2,3-disubstituted-2,3-dihydro-benzo[1,4]dioxin. An allyl was deduced from the correlations from H_2-1‴ (δ_H 3.36) to C-2‴ (δ_C 137.9), C-3‴ (δ_C 116.0), and the coupling from H_2-1‴ (δ_H 3.36) to C-5 (δ_C 117.4), C-6 (δ_C 134.0), C-7 (δ_C 122.0). Further, the correlations from H-2 to C-1″, C-2″,6″, C-3 and from H-3 to C-2, C-1′, C-2′,6′ showed the connectivities of C-2 to C-3, C-2 to C-1″, C-3 to C-1′.

The relative stereochemistry of **1** was determined by the coupling data and ROESY experiment (Figure 1). In ^{1}H NMR spectrum, H-2 (δ_H 4.81, d, J=15 Hz, 1H) and H-3 (δ_H 4.83, d, J=15 Hz, 1H) indicated *trans* stereochemistry. In ROESY spectrum, a strong cross-peak between H-2″ and H-2 along with the cross-peak between H-2′ and H-3 showed H-2β and H-3α or H-2α and H-3β. Therefore, the complete structure of **1** was ascertained as shown in Figure 1.[2,3]

Theoretical studies, at B3LYP/6-31G* level, indicate that the most favorable structure of **1** is as shown in Figure 1, from which one can realize that the two hydrogen atoms on bridge are of *trans* structure with the dihedral angle H(2)-C(2)-C(3)-H(3) being about 176°. In addition, the distance between H-2 and H-2″ is about 0.24 nm, which is in good agreement with our ROESY spectral results. Other structures, with the dihedral angle H(2)-C(2)-C(3)-H(3) being −55° and 76°, are even much less stable, and the relative energies are calculated to be about 10.08 and 20.16 kJ/mol (with zero-point energy correction) higher than that of **1** respectively. Exchanging position between groups –PhOH and –Ph$(OCH_3)_2$ has no energy difference. So, only the relative configuration can be determined, but the absolute configuration can not be determined. Figure 1 is only the figured diagram.

Experimental

General

Melting points were measured on a Beijing Ketai micromelting point apparatus and are uncorrected. Optical rotations were obtained on a Type AA-10R Automatic Polarimeter. IR spectra were recorded in KBr on a Nicolet AVATAR 360 FT-IR spectrophotometer. The ^{1}H (500 MHz, $CDCl_3$), ^{13}C (125 MHz, $CDCl_3$), and 2D NMR spectra were recorded on a Burker Ac-80&Ac-500 instrument using the signal of $CDCl_3$ as a reference (the singlet at δ 7.28 for the ^{1}H NMR data and a triplet centered at δ 77.4 for the ^{13}C NMR data). HRFABMS were recorded on a Bruker Daltonics Inc. APEX II FT-ICRMS spectrometer in the positive-ion mode using glycerol as the matrix. EIMS were collected on an HP-5988 or a ZAB-HS instrument at 70 eV by direct inlet. For column chromatography, Si gel (300—400 mesh, Yantai Yuanbo Chemical Ltd., Yantai, P.R. China) and Sephadex LH-20 were used. TLC was performed on precoated plates (Kieselgel GF_{254}, Yantai) with detection effected by UV light (254 nm) and using PMA.

Plant material

The resin, called ‘dragon’s blood’ of *Dracaena cochinchinensis* (Lour.) S. C. Chen was bought from Guangxi, P.R. China, in Oct. 2000, and identified by Prof. Pan Xuan, Chinese Academy of Medical Sciences.

Extraction and isolation

The resin (1.7 kg) was suspended in H_2O and the aqueous suspension was sequentially extracted six times respectively with petroleum ether, $CHCl_3$, and EtOAc (each, 1000 mL). After evaporation of the combined EtOAc extract, a red brown syrup (300 g) was yielded. This syrup was subjected to silica gel column chromatography, eluted with petroleum ether-EtOAc mixtures of increasing polarities, to obtain eight fractions (I—VIII). Fraction I was further separated by silica gel chromatography eluted with petroleum ether-EtOAc (15∶1, V∶V) to afford further five fractions, 1A, 1B, 1C, 1D, and 1E. Fraction 1A was purified on a Sephadex LH-20 column, by elution with MeOH/H_2O, to obtain 7-hydroxy-4'-methoxyflavane (**3**, 22 mg) (m.p. 138.5—140.2 ℃).[4] Using the silica gel column chromatography and Sephadex LH-20 separated fraction 1B, which afforded cochinchin, (2,3-*trans*)-6-allyl-2-(3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)-2,3-dihydrobenzo[1,4]dioxin (**1**, 15.2 mg). Fraction 1C was separated and purified by silica gel column chromatography to afford 4'-hydroxy-2,4-dimethoxydihydrochalcone (**5**, 30.5 mg) (m.p. 120.1—121.6 ℃).[5] Fraction II was rechromatographed on a silica gel column using petroleum ether-acetone (6∶1, V∶V) as the eluent to obtain 7-hydroxy-3-(4-hydroxybenzyl)chroman (**4**, 35 mg) (m.p. 124.6—126.1 ℃).[6] Fraction III was rechromatographed on a silica gel column using petroleum ether-acetone (6∶1, V∶V) as the eluent and purified with Sephadex LH-20 to obtain 4'-hydroxy-2,4,6-tri-

methoxydihydrochalcone (**6**, 27 mg) (m.p. 135.0—136.6 ℃).[5] Fraction IV was rechromatographed on a silica gel column using petroleum ether-EtOAc (4∶1, *V*∶*V*) as the eluent and purified with silica gel column to obtain 7,4'-dihydroxyflavone (**2**, 110 mg) (m.p.＞300 ℃).[7]

Cochinchin **(1)** Brown oil, $[\alpha]_D^{24}$ −30.2 (*c* 0.0053, CH_3COCH_3); ^1H NMR (500 MHz, $CDCl_3$,) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; IR (KBr) ν_{max}: 3390 (OH), 1597,1515, 1504, 1462 (double bond and aromatic ring), 1154, 1065 (ether) cm^{-1}; EIMS *m/z* (%): 404 $[M]^+$ (41.7), 256 $[M-C_3H_5-C_6H_4OH-2H]^+$ (100); HRFABMS *m/z*: 427.1513 $[M+Na]^+$, 404.1615 $[M]^+$ (calcd for $C_{25}H_{24}O_5$, 404.1618).

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