Two New Phenolic Compounds from the Heartwood of Caesalpinia sappan L.

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Abstract: Two new phenolic compounds, epicaesalpin J and 7,10,11-trihydroxydracaenone, were isolated from the heartwood of Caesalpinia sappan L. Their structures were identified by spectroscopic analysis methods, such as 1D and 2D NMR, along with the high resolution mass spectral data. The NO inhibition activities of two new compounds and six known compounds were tested.

Keywords: Caesalpinia sappan L.; dracaenone; epicaesalpin J; phenolic compounds

1. Introduction

Caesalpinia sappan L. (Leguminosae) is widely distributed in Thailand, Indonesia, Vietnam, Burma, India and South and Southwest China [1]. The dried heartwood of this plant, Sappan Lignum, is popularly used as a Traditional Chinese Medicine for the treatment of menorrhagia, cardiovascular and cerebrovascular diseases [2]. Previous investigations revealed that the extract of Sappan Lignum presented diverse and remarkable bioactivities, and therefore could be used as an anti-inflammatory, antimicrobial, antihypertensive and antiatherogenic agent. Prompted by the promising pharmaceutical properties, extensive studies on the phytochemical constituents of Sappan Lignum have been carried
out, which has resulted in the separation of various components including homoisoflavonoids [3–9], diterpenoids [10], dibenzoxocins [11–18], and a lactone [19]. As a part of our continuing studies on C. sappan [20,21], we report herein the isolation and structural identification of two new compounds 1 and 2 (Figure 1). Moreover, the NO inhibition activities of these new compounds and six known compounds 3–8 (Figure 1) were tested.

2. Results and Discussion

Compound 1 was obtained as a colorless gum, $[\alpha]_D^{25} +317.3$ (c 0.30, MeOH), UV $\lambda_{\text{max}}$ (MeOH) nm: 212, 242. Its molecular formula was determined as C$_{17}$H$_{16}$O$_6$ by HR-ESI-MS (found 317.1021 [M+H]$^+$, calcd. 317.1025). The IR spectrum of 1 showed $\alpha,\beta$-unsaturated ketone (1649 cm$^{-1}$), aromatic ring (1595 cm$^{-1}$) and hydroxyl group (3443 cm$^{-1}$) absorptions. The $^1$H- and $^{13}$C-NMR spectra of 1 displayed the characteristic signals of a methoxyl group ($\delta$ 3.62), an oxymethylene ($\delta$ 4.18 and 3.79), an
oxymethine (δ 3.48), an α,β-unsaturated ketone group, and a tetrasubstituted benzene ring, respectively. All protons and carbons were unambiguously assigned by 1D and 2D NMR experiments, including 1H-1H COSY, HSQC, and HMBC (Table 1, Figure 2). Comparison of the NMR data with those of the known compound, caesalpin J (3) [11,21], revealed that 1 possessed a similar skeleton. The major difference is the upfield shift of H-13 from δ 3.84 in 3 to δ 3.48 in 1, and the same phenomenon was also observed in the compounds haematoxin (10) and epihematoxin (9) [22], which suggested that 1 was a stereoisomer of 3. The absolute configuration of 3 had been established in [12] by an X-ray crystallographic study of its triacetate. According to this paper, the ring B and C in 3 were both in a chair conformation. The optical rotation of 1 (+371.3) was consistent with that of 3 (+445.0), which suggested that 1 had the same configuration. In the NOESY spectrum of 1, the cross peaks between H-13 and H-6β, and OMe-13 and H-8 unambiguously confirmed that 1 was a C-13 epimer of 3, and the methoxyl group at C-13 was α-oriented (Figure 3). Thus, the structure of 1 was fully elucidated, and it was named epicaesalpin J.

Table 1. 1H-NMR (500 MHz) and 13C-NMR (125 MHz) data for 1 and 2 (δ in ppm and J in Hz).

| No. | 1 (in DMSO-d6) | 1 (in CD3OD) | 2 (in CD3OD) |
|-----|---------------|--------------|--------------|
|     | δC | δH | δC | δH | δC | δH |
| 1   | 146.3 | 7.03 d (10.0) | 149.0 | 7.16 d (10.0) | 151.5 | 6.88 d (10.0) |
| 2   | 129.2 | 6.46 dd (10.0, 1.5) | 130.4 | 6.51 dd (10.0, 1.5) | 128.5 | 6.45 dd (10.0, 1.5) |
| 3   | 187.1 |  | 190.9 |  | 191.4 |  |
| 4   | 108.5 | 5.48 d (1.5) | 109.8 | 5.57 d (1.5) | 108.1 | 5.58 d (1.5) |
| 4a  | 175.0 |  | 178.6 |  | 179.7 |  |
| 6   | 77.8 | 4.12 d (11.0) | 79.8 | 4.18 d (11.0) | 81.0 | 3.88 dd (11.0) |
|     | 3.72 d (11.0) |  | 3.79 d (11.0) |  | 4.27 dd (11.0) |  |
| 7   | 69.4 | 71.3 | 66.8 |  |
| 8   | 37.6 | 3.16 d (16.0) | 39.0 | 3.30 d (16.0) | 43.5 | 3.16 d (16.0) |
|     | 2.83 d (16.0) |  | 2.88 d (16.0) |  | 3.10 d (16.0) |  |
| 8a  | 122.6 |  | 124.2 |  | 129.5 |  |
| 9   | 115.4 | 6.52 s | 116.6 | 6.59 s | 116.9 | 6.64 s |
| 10  | 145.0 |  | 146.6 |  | 146.8 |  |
| 11  | 143.6 |  | 145.2 |  | 145.1 |  |
| 12  | 112.9 | 6.26 s | 114.1 | 6.35 s | 113.4 | 6.44 s |
| 12a | 126.6 |  | 128.5 |  | 125.5 |  |
| 12b | 51.1 | 53.5 | 47.8 |  |
| 13  | 82.7 | 3.44 s | 84.8 | 3.48 s | 41.1 | 1.99 dd (11.5) |
|     |  |  |  |  | 2.34 dd (11.5) |  |
| OCH3 | 61.4 | 3.53 s | 62.6 | 3.62 s |  |  |

Compound 2 was obtained as a colorless gum, [α]D25 −152.6 (c 0.54, MeOH). Its molecular formula was determined as C16H14O5 by HR-ESI-MS (found 285.0765 [M−H]+, calcd. 285.0769). The 1H- and 13C-NMR data of 2 were similar to those of the known compound 7,10-dihydroxy-11-methoxydracaenone (11), except that 2 showed no methoxyl signals. Considering that the molecular weight of 2 was 30 Da less than that of 11, compound 2 was identified as 7,10,11-trihydroxydracaenone. According to [23], the absolute configuration of 11 was different from those of 3 and 1, and 11 had a C ring boat conformation. The optical rotation of 2 was −152.6, which was similar to that of 11 (−465.9). Moreover, in the NOESY
spectrum, the cross peaks between H-4 and H-6β, and H-8 and H-6α unambiguously confirmed that 2 had the same configuration as 11, which was opposite of that of 1 and 3 (Figure 3). All protons and carbons were unambiguously assigned by 1D and 2D NMR experiments, including 1H-1H COSY, NOESY, HSQC, and HMBC (Table 1, Figures 2 and 3). Thus, the structure of 2 was confirmed as 7,10,11-trihydroxydracaenone.

Figure 2. Key HMBC correlations of compound 1 and 2.

Figure 3. Key NOESY correlations of compounds 1 and 2.

The dracaenone skeleton is uncommon in natural products, and only six compounds with this type of skeleton have been reported [11,22,23]. They are isolated from Caesalpinia sappan L. [11], Haematoxylon campechianum [22], and Dracaena loureiri Gagnep [23], although it is not unexpected that they exist in different genera, because it is believed that the dracaenone skeleton is the oxidative cyclization product of homoisoflavan in biogenetic pathway [24], so we deduced that 1 and 2 should be the oxidative products of 12 and 13, respectively (Scheme 1).

Two new compounds and six known compounds caesalpin J (3), 1-hydroxy-7-methylxanthone (4), 1,5-dihydroxyxanthone (5), 1,7-dihydroxyxanthone (6), butein (7), and sappanone A (8) were evaluated for their inhibitory activities against nitric oxide production in LPS-activated BV-2 microglia according to a previously described method [25]. Compounds 4, 6, 7, and 8 showed obvious inhibitory activity, with IC50 values that were lower than those of quercetin, the positive control (Table 2).
Scheme 1. Proposed biogenetic pathway for 1 and 2.

Table 2. Inhibitory effects of compounds 1–8 and quercetin.

| Compound | Viability (at 50 µM, %) | IC₅₀ (µM) | Compound | Viability (at 50 µM, %) | IC₅₀(µM) |
|----------|------------------------|----------|----------|------------------------|----------|
| 1        | 96.45                  | 52.62    | 6        | 101.64                 | 21.46    |
| 2        | 99.29                  | 56.71    | 7        | 94.00                  | 15.46    |
| 3        | 96.13                  | 45.87    | 8        | 99.29                  | 8.26     |
| 4        | 98.94                  | 14.23    | quercetin|                        |          |
| 5        | 101.19                 | 28.65    |          |                        |          |

3. Experimental

3.1. General

Optical rotations were measured on an Autopol III automatic polarimeter (Rudolph Research Co., Hackettstown, NJ, USA). IR spectra were measured using a Thermo Nicolet Nexus 470 FT-IR spectrometer (Thermo Nicolet, Madison, WI, USA) with KBr disks. HR-ESI-MS were carried out on a Q-STAR ESI-TOF-MS/MS spectrometer (AB SCIEX, Framingham, MA, USA). 1D and 2D-NMR spectra were recorded on a Varian Inova-500 spectrometer (Varian, Palo Alto, CA, USA) with TMS as internal standard. Silica gel (200–300 mesh, Qingdao Marine Chemical, Qingdao, China) was used for column chromatography. Sephadex LH-20 gel was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). MCI gel (CHP 20/P120) was purchased from Mitsubishi Chemical Industries Ltd. (Tokyo, Japan). Semi-preparative HPLC was performed on a Waters XBridge semi-preparative C-18 column (10 × 250 mm, 5 µm, Waters Co., Milford, CT, USA), eluting with MeOH/H₂O at a flow rate of 2–3 mL/min; the detector used was DAD (200–400 nm) at room temperature. Fractions were monitored by TLC, and spots were visualized by spraying TLC plates with 10% sulfuric acid in ethanol and heating at 110 °C for 5–10 min.
3.2. Plant Material

Sappan Lignum (the heartwood of *Caesalpinia sappan* L.) was purchased from the Anguo medicinal materials market, Hebei Province of China, in September 2010. The plant material was authenticated by one of the authors, Prof. Peng-Fei Tu (School of Pharmaceutical Sciences, Peking University) and a voucher specimen (No.M-6-(5)) was deposited at the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

3.3. Extraction and Isolation

The dried heartwood of *Caesalpinia sappan* L. (21 kg) were chopped and extracted three times with 95% EtOH (168 L, 126 L, 126 L) to give 2.5 kg of crude extract. The extract was then suspended in water (5 L) and successively partitioned with petroleum ether, EtOAc and *n*-BuOH (20 L) to give after solvent removal fractions PE (60 g), EA (1,400 g), and BU (360 g), respectively.

A portion of EA (800 g) was subjected to silica gel column chromatography eluted with a step-wise gradient of CHCl₃ and MeOH to obtain fractions 1–12. Fraction 5 was chromatographed on silica gel eluted with petroleum ether/EtOAc (3:1 to 1:1) to give subfractions 5a–f. Fraction 5e was separated through Sephadex LH-20 eluted with CHCl₃/MeOH (1:1), and further purified by semi-preparative HPLC (MeOH/H₂O 85:15) to yield 1 (12.0 mg), along with 3 (10.0 mg). Fraction 8 was subjected to silica gel column chromatography eluted with CHCl₃/Me₂CO (10:1–1:1) to give 8a–h. Fraction 8d was subjected to a silica gel column eluted with CHCl₃/MeOH (20:1–5:1) to give 8da–df. Fraction 8db was separated by a MCI column eluted with MeOH/H₂O (30:70–100:0) to yield 2 (11.0 mg). The isolation of the known compounds had been reported in our previous papers [21,22].

3.4. Spectral Data

*Epicaesalpin J* (1). Colorless gum. [α]₂⁰⁻³⁷₁.₃° (c 0.30, MeOH), UV λmax (MeOH) nm: 212, 242. IR νmax (KBr) cm⁻¹: 3443, 2957, 1649, 1595, 1454, 1395, 1016. HR-ESI-MS m/z: 317.1021 [(M+H)⁺, calcd. for C₁₇H₁₇O₆ 317.1025]. ¹H-NMR and ¹³C-NMR (CD₃OD and DMSO-d₆) see Table 1.

*7,10,11-Trihydroxydracaenone* (2). Colorless gum. [α]₂⁰⁻₁₅₂.₆° (c 0.54, MeOH). UV λmax (MeOH) nm: 214, 240. IR νmax (KBr) cm⁻¹: 3381, 1653, 1591, 1522, 1451, 1395, 1065. HR-ESI-MS m/z: 285.0765 [(M−H)⁻, calcd. for C₁₆H₁₄O₅ 285.0769]. ¹H-NMR and ¹³C-NMR (CD₃OD) see Table 1.

The spectral data of known compounds were reported in our previous literatures [20,21].

3.5. Inhibition of NO Production in LPS-Stimulated BV-2 Microglia

The assay was performed according to a previously described method [25]. Each compound was dissolved in DMSO and further diluted in the medium to produce different concentrations with a maximum concentration of 50 μM. The absorbance was measured at 570 nm with a Multilabel Plate Reader (Sunrise TECAN, Männedorf, Switzerland). Cytotoxicity was determined with the MTT assay. Quercetin (Sigma-Aldrich, Foster City, CA, USA) was used as the positive control.
4. Conclusions

The chemical study of the heartwood of *C. sappan* resulted in the isolation of two new compounds: epicaesalpin J (1) and 7,10,11-trihydroxydracaenone (2). Compounds 1 and 2 both have the dracaenone skeleton, which is uncommon in natural products. The new compounds, together with six known phenolic compounds, were evaluated for NO production inhibitory activity in LPS-stimulated BV-2 microglia. Compounds 4, 6, 7, and 8 showed obvious inhibitory activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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