Bioactive sesquiterpenes isolated from the essential oil of Dalbergia odorifera T. Chen

Yi Tao, Yi Wang *

Institute of Pharmaceutical Informatics, Zhejiang University, Zijingang Campus, No. 388 Yuhangtang Rd, Hangzhou 310058, PR China

A R T I C L E   I N F O
Article history:
Received 26 June 2009
Accepted in revised form 26 November 2009
Available online 4 December 2009

Keywords:
Dalbergia odorifera
Antithrombotic
Anti-platelet
Sesquiterpenes

A B S T R A C T
Investigation of the essential oil from the heartwood of Dalbergia odorifera T. Chen afforded the sesquiterpenes 1 and 2, that both showed anti-platelet activity. Their backbones were totally the same only with subtle difference in the chiral centre. Additionally, antithrombotic and anti-platelet activities of these two compounds were measured. The results showed that the antithrombotic activity of these two compounds was poor, while their anti-platelet activity was strong at middle and high concentrations.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Lignum Dalbergia odorifera (Chinese name Jiangxiang), a dried heartwood of the trunk and root of D. odorifera T. Chen (Fam Leguminosae), is an important Chinese wood [1], that has been in widespread use in various Chinese herbal preparations, such as Danshen injection, Qi-Shen-Yi-Qi decoction, and Guanxin-Dan-shen pills and so forth. In China, the essential oil of D. odorifera is regarded as a useful treatment of cardiovascular diseases, and previous reports indicated that it can prevent the occurrence of myocardial infarction [2]. However, the chemicals that contributed to the cardioprotective effects of this wood are still not fully understood yet.

According to the previous reports, volatile oil [3] and flavonoids [4–8] are major components in D. odorifera. These reports showed that the essential oil of D. odorifera chiefly comprised trans-nerolidol, β-bisabolene and trans-β-farnesene [3]. But the bioactivity of those compounds was rarely studied. The aim of present study is to isolate the major constituents from the essential oil of D. odorifera and to evaluate their bioactivity. Through GC–MS analysis combined with NMR determination, it was found that trans-nerolidol, compounds 1 and 2 are the main constituents of the essential oil. By searching related literatures, we found that the 1H NMR and GC–MS data of compounds 1 and 2 were in good agreement with the data of synthesized compounds 5d and 5a in Kaiser and Lamparsky’s report [9]. They were also reported in Hedychium gardnerianum Roscoe [10], Myrceugenia cocculata [11] and Teucrium royleanum Wall. ex Benth [12]. Although these two compounds were not new in their chemical structures, but they were separated from the D. odorifera for the first time. Moreover, antithrombotic and anti-platelet activities of these two sesquiterpenes were evaluated.

2. Experimental

2.1. General

TLC: Merck precoated plates (silica gel 60 F254) of 0.25 mm thickness. TC-15 electrothermal mantle. BSZ-100 automated device for collecting eluted fraction. GC–MS: Agilent 6980 GC system equipped with a fused-silica capillary column chemically (30 m × 0.25 mm i.d.) bonded with 0.25 μm ZB-5MS stationary phase (Phenomenex) and Agilent 5973 mass selective detector. 1H NMR, 13C NMR, 1D and 2D NMR (HSQC, HMBC, COSY, and NOESY) Spectra: Bruker Avance III 500 MHz
NMR spectrometer; $\delta$ in ppm, $J$ in Hz. ESI-MS: LCQ-DECA-Thermo-Finnigan system equipped with a hot ESI source IR: JascoFT-IR4100. The kits for determination of activated partial thromboplastin time (APTT), thrombin time (TT), and prothrombin time (PT) were commercially obtained from Shanghai Sun Biotech. Co. ADP was obtained from Sigma-Aldrich. The anti-platelet instruments and related accessories were purchased from the Precil Co.

2.2. Plant material

The heartwood of *D. odorifera* T. Chen was collected in Guangxi Province, P.R. China, in January 2007 and identified by Dr. Wu Bin. A voucher specimen (No. 070120) was deposited with the Institute of Pharmaceutical Informatics, Zhejiang University, P.R. China.

2.3. Extraction and isolation

An accurately weighed amount (500 g) of sample decoction pieces was suspended in 4 L of distilled water and stood overnight at room temperature. The extraction was performed in an electothermal mantle using an essential oil extracted apparatus with the essential oil collected once an hour for 6 h. When the extraction was finished, the essential oil was combined together. An aliquot of 3.9 ml essential oil was obtained and stored in the refrigerator at 4 °C for further experiment. The essential oil (0.93 g) was first chromatographed on a silica gel column and subjected to isocratic elution with petroleum ether/AcOEt (100:1 (v/v)). The elute was collected with test tubes (20 ml) on automated device for fraction collection and then analyzed by GC–MS.

Elute from No. 171–179 tubes mainly consisted of compound 1 (95% purity), so they were combined together (92 mg). The rest of the part were subjected to gradient elution with ether/AcOEt (49:1,19:1,9:1,4:1 (v/v)) to yield four fractions. Based on GC–MS analysis, it was found that Frc.4 mainly contains compound 2 (98% purity, 120 mg).

**Compound 1:** (3S,6S,7R)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol. 1 Yellowish oil. $[\alpha]_D^{22} + 68$ ($c = 0.001$, CHCl₃). IR: 3273, 2925, 1638, 1451, 1375, 1081, 985, and 917 cm⁻¹. 1H and 13C NMR: see Fig. 1, Table 1 and Supplementary data. ESI-MS: 239.36 ([M+H]+). GC–MS: 155 (5), 138 (14), 127 (10), 111 (20), 109 (100), 93 (27), 81 (13), 69 (57), 55 (25), 43 (41), and 41 (32).

**Compound 2:** (3S,6S,7R)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol. 2 Yellowish oil. $[\alpha]_D^{22} + 46$ ($c = 0.001$, CHCl₃). IR: 3458, 2972, 2926, 1647, 1454, 1375, 1057, 997, and 917 cm⁻¹. 1H and 13C NMR: see Fig. 1, Table 2 and Supplementary data. ESI-MS: 239.36 ([M+H]+). GC–MS: 155 (4), 138 (30), 127 (7), 111 (28), 109 (100), 93 (28), 81 (26), 69 (68), 55 (40), 43 (87), and 41 (56).

2.4. Antithrombotic assay

A total of 3 normal male SD rats (550 ± 10 g) were purchased from Zhejiang University Laboratory Animal Centre (ZJULAC, Hangzhou, China). After an acclimation period for 3 days, 9.5 ml of blood was obtained from the abdominal aorta of these rats and transferred to a 10-ml plastic tubes containing 1/10 volume of 0.109 mol/L sodium citrate solution. Blood samples were centrifuged at 3000 rpm for 15 min and the supernatants were collected. The group assignments were as follows: six treatment groups received the solution of compound 1 and compound 2 with the help of Tween 20 (0.05 ml/10 ml) to yield a gradient concentration of 1 μg/ml, 10 μg/ml, and 100 μg/ml in serum. The control group was added to the same volume of saline with identical amount of Tween 20 (0.05 ml/10 ml). After thoroughly vortexing, TT, PT and APTT of the serum were determined by the kits according to the supplier’s instructions. The animal experiment was carried out under the guidelines for Animal Experiment of Zhejiang University (Hangzhou, China) and the protocol was approved by the Animal Ethics Review Committee of Zhejiang University.

2.5. Anti-platelet assay

A total of 15 normal male SD rats (250 ± 10 g) were purchased from Zhejiang University Laboratory Animal Centre (ZJULAC, Hangzhou, China). After an acclimation period for 3 days, 75 ml of blood was obtained from the abdominal aorta of these rats and transferred to plastic tubes containing 1/10 volume of 0.109 mol/L sodium citrate solution. Blood samples were firstly centrifuged at 900 rpm for 10 min and the supernatants were collected as PPP (platelet poor plasma). Second, the remainder was centrifuged again at 4000 rpm for 10 min and the supernatants were obtained as PRP (platelet rich plasma). The next anti-platelet assay was performed according to the instructions of Precil’s manual. Test groups were added to the solutions of compounds 1 and 2 to yield a gradient concentration of 1 μmol/ml, 5 μmol/ml, and 10 μmol/ml in serum, respectively. All experiments were performed for three times.

2.6. Chirality determination procedure

A convenient Mosher ester procedure [13,14] was employed to determine the absolute configuration of compound 1 and compound 2, both of which have a chiral centre located in the C7. Compound 1 (0.9 mg), dried completely under oil pump, was dissolved in a 500 μL aliquot of pyridine-d5

![Fig. 1](image-url)