

CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *ZANTHOXYLUM SCANDENS*MING-JEN CHENG^{1,2,3} CHUAN-FANG LIN^{1,3} HSUN-SHUO CHANG¹, IH-SHENG CHEN^{1*}¹Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China.²M.-J. C. and C.-F. L. contributed equally to the work in this paper.

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ABSTRACT

One new dioxoaporphine alkaloid, zanthodione (**1**), together with nineteen known compounds have been isolated from the stem bark of *Zanthoxylum scandens*. The structures of these compounds were determined by means of spectral analyses. Except for zanthodione (**1**), methyl vanillate, vanillic acid, and 2-(4'-hydroxyphenyl)ethyl hexacosanoate, all of the other 16 compounds, as same constituents found in *Z. ailanthoides*, which were evaluated for inhibition of HIV replication in H9 lymphocyte cells in our previous report in *Bioorg. Med. Chem.*. The reported decarine, γ -fagarine, and (+)-platydesmine were the most potent anti-HIV constituents, with EC₅₀ values ranging from 0.1 to 1.34 μ g/mL and TI (Therapeutic Index) values from 74 to 231, also isolated from the stem bark of this plant.

Keywords: *Zanthoxylum scandens*, Rutaceae, stem bark; dioxoaporphine; anti-HIV activity.

INTRODUCTION

Zanthoxylum scandens Blume (*Zanthoxylum cuspidatum* Champ. ex Benth.) (Rutaceae) is a woody climbing vine distributed in India, China, the Ryukyus, Sumatra, Java, and Borneo, growing at low altitudes throughout Taiwan.¹ The root and stem with leaves of this plant have been used as folk medicine to treat abdominal pain, toothache, rheumatism, and traumatic injury.² The chemical constituents of this plant's (leaves,³ root bark,³ wood,^{4,5} root,⁶ and bark,^{5,7,8}) such as flavonoids, benzo[*c*]phenanthridines, phenylpropanoids, coumarins, quinolines, *N*-isobutylamides, lignans, triterpenoids, indolopyridoquinazoline s, and others have been reported in the past. So far, twenty-eight constituents have been obtained from this plant. Nonetheless it may be assumed that minor constituents still remain to be found.

In our continuing studies on the anti-HIV constituents of Formosan plants, over 1000 species have been screened for *in vitro* anti-HIV activity to date, and *Z. scandens* has been found to be one of the active species with a therapeutic index (TI, TI value = concentration that inhibits uninfected H9 cell growth by 50 %/concentration that inhibits viral replications by 50 %) of >200, as in the case of the root bark of *Z. ailanthoides*⁹ and the root wood of *Z. integrifolium*.¹⁰ Careful re-examination of the stem bark of *Z. scandens* led to the isolation of the new compound, zanthodione (**1**), along with 19 reported known compounds, 10 of which had not been described previously as constituents of this plant. The anti-HIV principles of *Z. ailanthoides* already certified to be decarine, γ -fagarine, and (+)-platydesmine, with TI values >226, >231, and >74.4,⁹ respectively. These three bioactive compounds also isolated in this study. In this article, structural elucidation of **1** and the SAR (structure and activity relationship) on the anti-HIV activity of some of the isolates compounds are discussed herein.

EXPERIMENTAL

General Experimental Procedures

Melting points were determined with a YANACO micro-melting point apparatus and were uncorrected. IR spectra were taken on a Hitachi 260-30 spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EIMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS were recorded on a JEOL JMX-HX 110 mass spectrometer. ¹H NMR and ¹³C NMR spectra were measured on a Varian Gemini 200, Varian Unity Plus 400, and Varian Unity Inova 500 and 600 spectrometers, and are given in parts per million (δ) downfield from internal TMS. Silica gel 60 (Merck 70-230 mesh, 230-400 mesh) was used for column chromatography, and Si gel 60 F₂₅₄ (Merck) for TLC.

Plant Material

The stem bark of *Z. scandens* was collected in Mutan, Pintung County, Taiwan, 22°17'N, 120°81'E in July, 2003. A voucher specimen (Chen 5528) was deposited in the Herbarium of the College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and separation of compounds

The stem bark (7.6 kg) was sliced into chips, extracted with cold MeOH and concentrated under reduced pressure. The methanolic extract (380 g) was partitioned between CHCl₃ and H₂O (1:1), to afford CHCl₃ (fraction A, 90 g) and H₂O-soluble fractions, and an insoluble precipitate (fraction B, 5 g). The H₂O solution was extracted with *n*-BuOH. The *n*-BuOH extract was concentrated *in vacuo* to give a residue (fraction C, 14 g). The detail separation and fractionation of fractions A, B, and C were illustrated by the following three schemes.

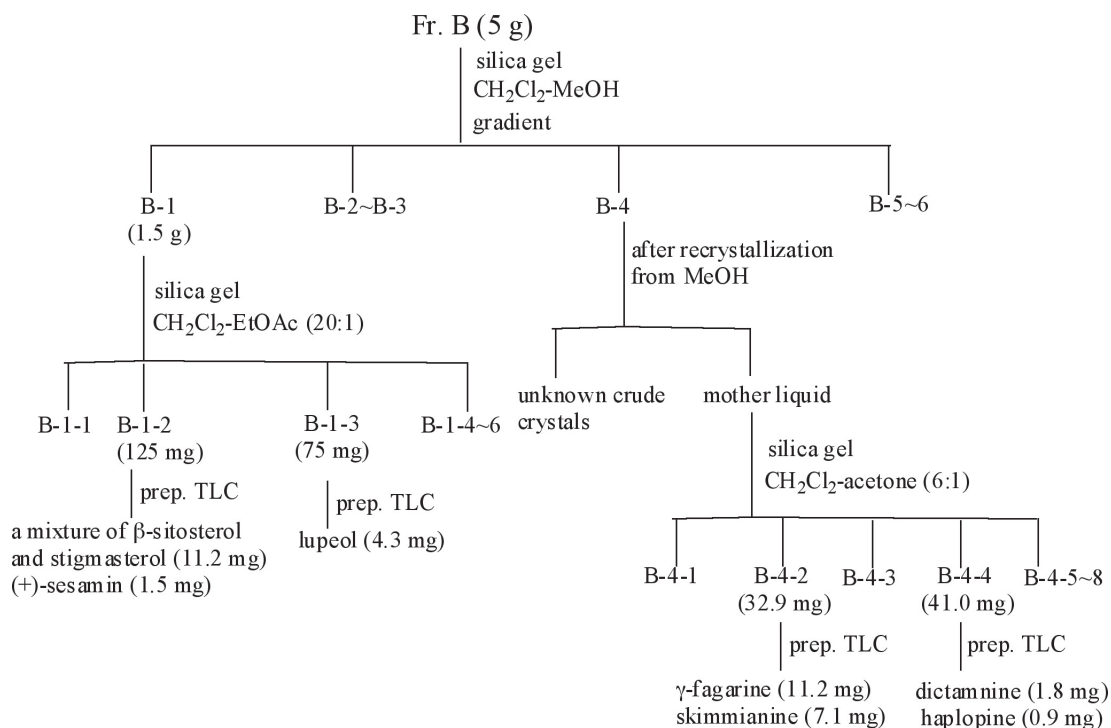
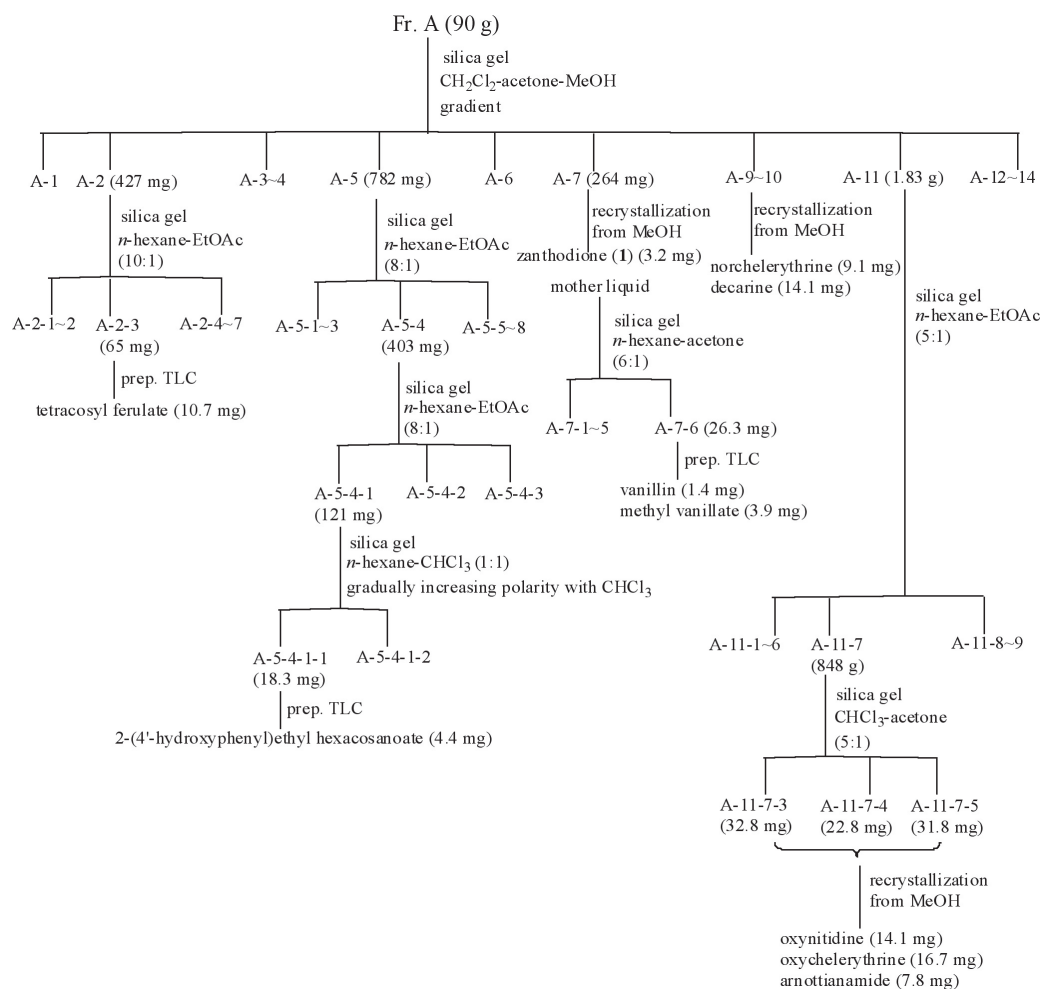
Spectroscopic data

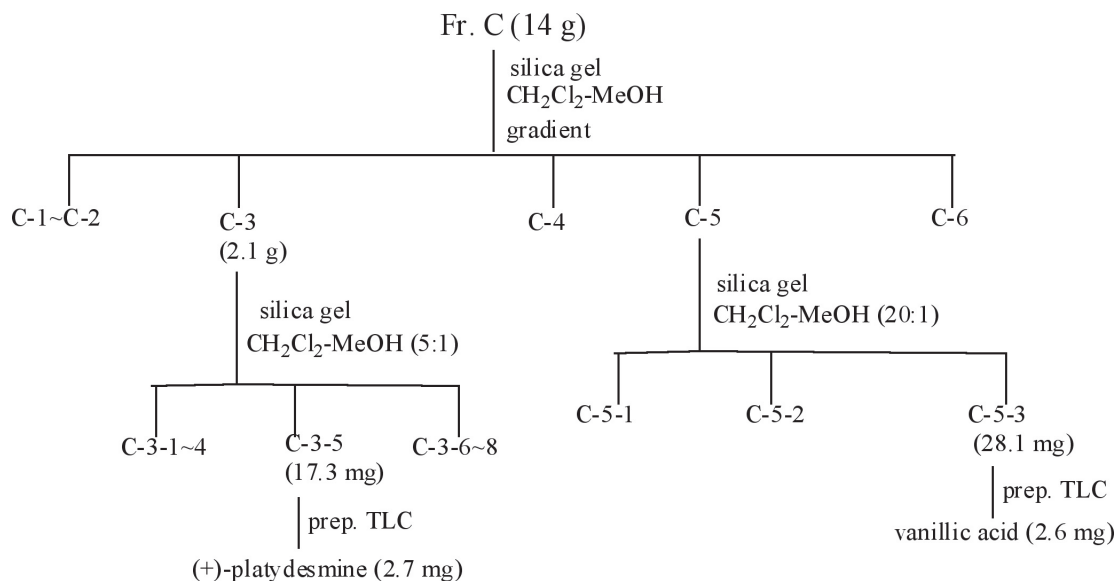
Zanthodione (1): orange needles; mp. 254-256 °C; UV (MeOH) λ_{\max} (log ϵ) 239 (sh) (4.49), 249 (4.51), 392 (sh) (3.58), 408 (4.51), 428 (3.58) nm; IR (KBr) ν_{\max} 3389 (OH); 1660, 1646 (C=O); 1601, 1460 (aromatic ring C=C); 1040, 940 (OCH₂O) cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) δ 6.40 (2H, s, OCH₂O), 7.73 (1H, br ddd, *J* = 8.4, 7.2, 1.8 Hz, H-10), 7.77 (1H, dt, *J* = 7.2, 1.2 Hz, H-9), 8.04 (1H, s, H-7), 8.26 (1H, s, NH exchangeable with D₂O), 8.29 (1H, dd, *J* = 7.2, 1.8 Hz, H-8), 9.26 (1H, d, *J* = 8.4 Hz, H-11), 11.98 (1H, s, OH exchangeable with D₂O); EIMS *m/z* 307 [M]⁺ (90); HRESIMS *m/z* 307.1481 (calcd for C₁₇H₉NO₅, 307.1480).

RESULTS AND DISCUSSION

Zanthodione (**1**) was obtained as orange needles. Its molecular formula C₁₇H₉NO₅ was established by EIMS ([M]⁺, *m/z* 307) and HRESIMS. The UV spectrum showed absorption maxima at 239 (sh), 240, 249, 392 (sh), 408, 428 nm, that indicated the presence of a 4,5-dioxoaporphine skeleton.^{11,12} Its IR spectrum indicated the presence of OH/NH (3000-3389 cm⁻¹), two aryl ketone C=O (1660, 1646 cm⁻¹), one methylenedioxy (1040, 940 cm⁻¹) and a six-membered lactam (1587 cm⁻¹) functionalities. The ¹H NMR spectrum revealed five aromatic protons, two D₂O exchangeable singlets of a H-bonded phenolic hydroxyl [δ 11.98 (1H, s)], and an amidocarbonyl proton at δ 8.26 (1H, s), and a methylenedioxy group at δ 6.40 (2H, s). Five aromatic protons of the spectrum closely resembled those of other 7,8,9,10,11-unsubstituted dioxoaporphine.^{11,12} Therefore, the methylenedioxy group had to be on ring A. The singlet at δ 8.04 (1H, s) was ascribed to H-7 while H-8, H-9, H-10 and H-11 appeared as a complex ABCX coupling system. In agreement with the spectra of other C-11-unsubstituted aporphines, dehydro- and oxoaporphines, the most downfield doublet [δ 9.26 (1H, s)] was characteristic of H-11, the 2H [δ 7.73 (1H, ddd, *J* = 8.4, 7.2, 1.8 Hz), 7.77 (1H, dt, *J* = 7.2, 1.2 Hz)] were assigned to H-10, 9, and another proton at δ 8.29 (1H, dd, *J* = 7.2, 1.8 Hz) was due to H-8. The correlations of H-7/NH, and H-8/H-7, 9 were also disclosed in the NOESY experiment (Fig. 1) and further supported the position of above assignments. By means of ¹H-NMR, ¹H,¹H-COSY, and NOESY (Fig. 1) data, the structure of zanthodione was shown to be 4-hydroxy-7H-benzo[*g*][1,3]dioxolo[4',5':4,5]benzo[1,2,3-*de*]quinoline-5,6-dione (**1**).

Dioxoaporphine alkaloids are unusual in nature and have been isolated from *Piper* (Piperaceae),¹³ *Aristolochia* (Aristolochiaceae),¹⁴ *Houttuynia*





(Saururaceae),¹⁵ *Fissistigma* (Annonaceae),¹⁶ and *Peperomia* (Piperaceae)¹⁷ species. This type alkaloid possessed antiplatelet aggregation activity¹⁶ and cytotoxicity activity¹⁸ in previous reports.

The known compounds included five benzo[*c*]phenanthridines: decarine,¹⁹ norchelerythrine,¹⁹ oxynitidine,¹⁹ oxychelerythrine,¹⁹ and arnottianamide,¹⁹ five

activity. From the results of the anti-HIV tests, the following conclusions can be drawn regarding these isolates: (a) The benzo[*c*]phenanthridine alkaloid, decarine, showed the highest anti-HIV activity in acutely infected H9 cells, with an EC₅₀ value of <0.1 μg/mL. It inhibited uninfected H9 cell growth with an IC₅₀ value of 22.6 μg/mL, giving a calculated TI value of >226.⁹ (b) In comparison with the inactive similar benzo[*c*]phenanthridine alkaloid, norchelerythrine, the 8-OH group in benzo[*c*]phenanthridine alkaloid seems to play an important role in the anti-HIV activity. The furoquinoline, γ-fagarine also showed a potent anti-HIV activity with EC₅₀ and TI value of <0.1 μg/mL and >231. In comparison, the other furoquinoline haplopine, which has a 7-OH in ring A, showed decreased activity, while replacing the 7-OH with a 7-OCH₃ group and no substituents in ring A gave skimmianine and dictamine, which showed no activity. We can conclude that the presence of the 8-OCH₃ group in furoquinoline may be essential for anti-HIV activity. Thus, among the above bioactive compounds, decarine, γ-fagarine and (+)-platydesmine, also re-isolated in this study, can be proposed as anti-HIV constituents of the stem bark of *Z. scandens*.

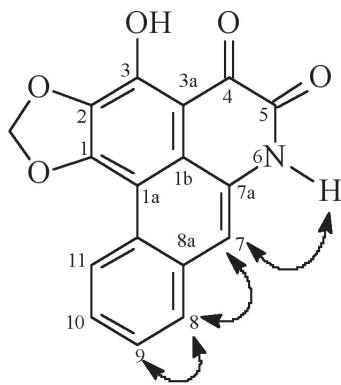
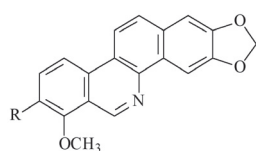


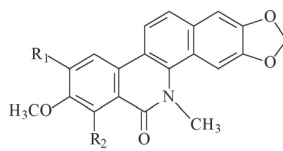
Fig. 1. Key NOESY correlations (↔) of **1**

furoquinolines: dictamine,²⁰ skimmianine,²⁰ γ-fagarine,¹⁹ (+)-platydesmine;¹⁹ and haplopine;²⁰ five benzenoids: vanillin,²¹ methyl vanillate,²¹ vanillic acid,²² tetracosyl ferulate,²³ and 2-(4'-hydroxyphenyl)ethyl hexacosanoate;²⁴ one lignan: (+)-sesamin;²⁵ two steroids: β-sitosterol,²¹ and stigmasterol;²¹ one terpenoid: lupeol.²⁶ These compounds were identified by comparison of their spectral data (UV, IR, ¹H NMR, MS) with the data from the corresponding values in the literature, or with authentic samples. Among them, vanillin, oxychelerythrine, oxynitidine, tetracosyl ferulate, vanillic acid, methyl vanillate, 2-(4'-hydroxyphenyl)ethyl hexacosanoate, (+)-sesamin, β-sitosterol, and stigmasterol were isolated for the first time from this plant.

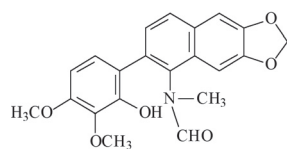
Zanthodione (**1**), methyl vanillate, vanillic acid, and 2-(4'-hydroxyphenyl)ethyl hexacosanoate, cannot be afforded the anti-HIV screening in this study due to their small quantities and exhausted. The other 16 known compounds isolated in this study, also found in *Z. ailanthoides*, have been screened for anti-HIV activity in our previous study.⁹ Except for decarine, γ-fagarine and (+)-platydesmine, the other 12 compounds show no anti-HIV



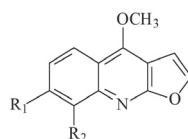
decarine R=OH
norchelerythrine R=OCH₃



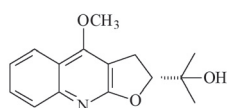
oxychelerythrine R₁=H, R₂=OCH₃
oxynitidine R₁=OCH₃, R₂=H



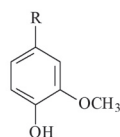
amottianamide



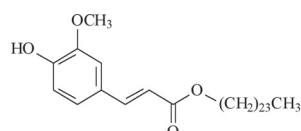
dictamnine R₁=R₂=H
skimmianine R₁=R₂=OCH₃
γ-fagarine R₁=H, R₂=OCH₃
haplopine R₁=OH, R₂=OCH₃



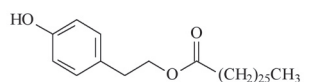
(+)-platydesmine



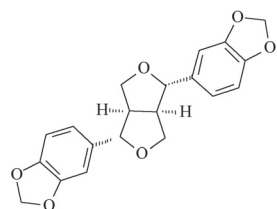
vanillin R=CHO
methyl vanillate R=COOCH₃
vanillic acid R=COOH



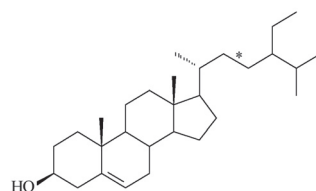
tetraacyl ferulate



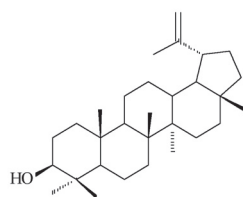
2-(4-hydroxyphenyl)ethyl hexacosanoate



(+)-sesamin



β-sitosterol
stigmasterol * = Δ



lupeol

ACKNOWLEDGEMENTS

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