A New Phenylpropanoid Ester from the Bark of *Zanthoxylum scandens* (Rutaceae)

Quang An Nguyen[a], Hanh Trinh Van-Dufat[a], Sylvie Michel[a], François Tillequin[a,*], Vincent Dumontet[b] and Thierry Sévenet[b]

[a] Laboratoire de Pharmacognosie de l'Université René Descartes, U. M. R./C. N. R. S. N° 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75006 Paris, France. E-mail: tillequi@pharmacie.univ-paris5.fr

[b] Institut de Chimie des Substances Naturelles du C. N. R. S., 91190 Gif-sur-Yvette, France

* Author for correspondance and reprint requests

Z. Naturforsch. **57 c**, 986–989 (2002); received July 15/August 2, 2002

Zanthoxylum scandens, Alkaloids, Phenylpropanoids, (*E*)-*O*-Geranylconyferyl Alcohol (9*Z*, 12*Z*)-linoleate

The alkaloids norchelerythrine, magnoflorine and (–)(S)-*O*-methylbalfourodinium cation were isolated from *Zanthoxylum scandens* bark collected in Vietnam, together with the flavanone glycoside hesperidin and the phenylpropanoids (*E*)-*O*-geranylconiferyl alcohol and (*E*)-*O*-geranylconiferyl alcohol (9*Z*, 12*Z*)-linoleate. This latter is a novel compound whose structure was elucidated on the basis of its spectral data and confirmed by chemical correlation.

Introduction

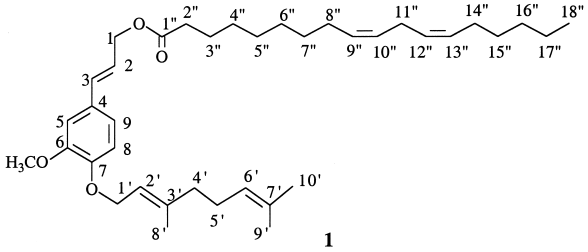
The genus *Zanthoxylum* L. (Rutaceae) includes some 250 species of trees and shrubs, with a worldwide, but predominantly tropical distribution (Engler, 1896; 1931). Morphologically, it is the only truly choricarpous genus in the Rutaceae, with fully free and stalked carpels (Gut, 1966). The very unspecialized flower morphology and vascular supply suggest a primitive position within the family (Moore, 1936; Das Graças *et al.*, 1988). Previous chemical studies on the genus have shown it to be a rich source of secondary metabolites (Waterman and Grundon, 1983). The Vietnamese flora includes some twelve *Zanthoxylum* species (Guillaumin, 1946; Pham Hoang, 2000), which have not been thoroughly studied from a chemical point of view. This prompted us to examine the secondary metabolites of these plants, in a continuation of our studies on Rutaceae (Doan Thi Mai *et al.*, 2001).

Zanthoxylum scandens Blume (= *Zanthoxylum cuspidatum* Champ.) is a climbing medium-sized shrub widely distributed throughout south-east Asia. Previous chemical investigations on this species were carried out on samples collected in Hong Kong (Arthur *et al.*, 1956; Brader *et al.*, 1993) and Taiwan (Ishii *et al.*, 1976; Ishii and Ishikawa, 1976). The wood and bark of formosan origin yielded alkaloids, including benzo[*c*]phenanthridines and related amides, aporphines, 2-quinolinones, and furo[2,3-*b*]quinolines, together with the phenylpropanoid cuspidiol (Ishii *et al.*, 1976; Ishii and Ishikawa, 1976). The roots of material from Hong Kong also afforded 2-quinolinones (Brader *et al.*, 1993), whereas hesperidin was isolated from the bark (Arthur *et al.*, 1956). In our hands, the chemical contents of the bark collected in Vietnam proved somewhat different, and we report here the isolation and structure determination of a novel phenylpropanoid ester, (*E*)-*O*-geranylconiferyl alcohol (9*Z*, 12*Z*)-linoleate, together with the identification of three alkaloids, a flavonoid glycoside, and a phenylpropanoid.

Results and Discussion

Six secondary metabolites were isolated from the CH_2Cl_2 and MeOH extracts of *Zanthoxylum scandens* bark. Three alkaloids were identified with the benzophenanthridine norchelerythrine (Krane *et al.*, 1984), the quaternary aporphine magnoflorine (Guinaudeau *et al.*, 1975; Slavik and Dolejs, 1973), and the quaternary furoroquinoline (–)(S)-*O*-methylbalfourodinium cation (Rideau *et al.*, 1979; Rapoport and Holden, 1959). Neutral products included the flavanone glycoside hesperi-

din (Okamura *et al.*, 1994), the phenylpropanoid (*E*)-*O*-geranylconiferyl alcohol (Shibuya *et al.*, 1992; Jen *et al.*, 1993) and the novel phenylpropanoid ester (*E*)-*O*-geranylconiferyl alcohol (9*Z*, 12*Z*)-linoleate.



1

(*E*)-*O*-Geranylconiferyl alcohol (9*Z*, 12*Z*)-linoleate (**1**) was obtained as a white amorphous product. The empirical formula was determined by accurate mass measurement as $C_{38}H_{58}O_4$. The UV spectrum recorded in MeOH showed absorption maxima attributable to a conjugated aromatic ring. The IR spectrum showed characteristic bands accounting for an ester group at 1736 and 1163 cm^{-1}. The ^1H NMR spectrum displayed three typical sets of signals. A first series of resonances was associated with a 3,4-disubstituted (*E*)-cinnamyl alcohol unit (δ 6.89, 1H, d, *J* = 1 Hz; δ 6.84, 1H, dd, *J* = 8 and 1 Hz; δ 6.75, 1H, d, *J* = 8 Hz; 6.53, 1H, d, *J* = 16 Hz; 6.12, 1H, dt, *J* = 16 Hz, *J* = 7 Hz; 4.76, 2H, d, *J* = 7 Hz) and one aromatic methoxyl group (δ 3.82, 3H, s). A second series accounted for a geranyloxyl substituent (δ 1.55, 1.64, and 1.68, 3 × 3H, 3s; 2.00 and 2.07, 2 × 2H, 2 m; 4.55, 2H, d, *J* = 7 Hz; 5.04, 1H, t, *J* = 7 Hz; 5.48, 1H, t, *J* = 7 Hz). A third series, including aliphatic signals accounting for 27H between 0.86 and 2.75 ppm and a 4H multiplet centered at 5.31 ppm was suggestive of a fatty acid ester chain including two double bonds. A thorough study of the ^{13}C NMR spectrum, in which typical CH resonances at δ 128.0, 128.1, 130.0, and 130.1 exhibited correlations with the 4H multiplet at 5.31 ppm in HMQC, permitted to ascribe the structure of (9*Z*, 12*Z*)-linoleic acid to the esterifying unit, in good agreement with literature data (Lie Ken Jie and Mustafa, 1997). The assignement of the methoxyl and geranyl moities on the aromatic ring was carried out using multi-impulsional NOESY experiment, which showed a typical correlation between H-5 at 6.89 ppm and O-CH_3 at 3.82 ppm. These data permitted to depict the structure of the novel phenylpropanoid as **1**. Structure **1** was finally confirmed by chemical correlation. Indeed, methanolysis of **1** afforded methyl (9*Z*, 12*Z*)-linoleate identical with an authentic sample and (*E*)-*O*-geranylconiferyl alcohol, whose spectral data were identical with those previously published (Shibuya *et al.*, 1992; Jen *et al.*, 1993).

It is interesting to note that the secondary metabolites isolated from *Zanthoxylum scandens* collected in Vietnam significantly differ from those obtained from samples of the same species collected in Hong Kong (Arthur *et al.*, 1956; Brader *et al.*, 1993) and Taiwan (Ishii *et al.*, 1976; Ishii and Ishikawa, 1976). Nevertheless, the major products isolated belong in all cases to the same chemical series and only the substitution pattern differs depending on the geographical origin. From a chemotaxonomic point of view, the isolation of phenylpropanoids together with that of alkaloids belonging to the benzophenanthridine, aporphine, and furoquinoline series indicates a lack of high skeletal specialization in the biosynthesis of *Zanthoxylum scandens* secondary metabolites. It clearly accounts for the primitive position of the genus *Zanthoxylum* in the Rutaceae family, in full agreement with a recent evolutionary interpretation of the family (Das Graças *et al.*, 1988).

Experimental

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers, using electrospray ionization (ESI-MS; Vc = 30 V). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade EtOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets on a Nicolet 510 FT instrument. 1H-NMR (δ [ppm], *J* [Hz]) and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz respectively, using a Bruker AVANCE-400 spectrometer. Multi-impusional 2D NMR experiments ($^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^{13}C-^1H$ HMQC, and $^{13}C-^1H$ HMBC) were performed using standard Bruker microprograms. Column chromatographies were carried out with silica gel 20–45 μm. Flash column chromatographies were conducted

using silica gel 60 Merck (35–70 μm) with an overpressure of 300 mbar (Still *et al.*, 1978). Microanalyses were in agreement with calculated values ± 0.4%.

Plant material

Bark of *Zanthoxylum scandens* Blume was collected at Pà Co, Mai Châu (Hoà Binh, Vietnam), on 5 April 1996. A voucher sample (VN 085) is kept in the herbarium of the Institute of Ecology of the National Center for Science and Technology in Hanoi, Vietnam.

Extraction and isolation

Dried, pulverized bark of *Zanthoxylum scandens* (300 g) was extracted successively with CH_2Cl_2 (4 × 0.5 l) and MeOH (6 × 0.5 l) at room temperature. The solvents were removed under reduced pressure to give crude CH_2Cl_2 and MeOH extracts (4.5 g and 6 g, respectively). An aliquot of the CH_2Cl_2 extract (2 g) was subjected to flash column chromatography on silica gel, using a CH_2Cl_2-MeOH gradient of increasing polarity to yield 9 fractions. Further column chromatographies on silica gel 20–45 μm, performed on fractions 2 to 6 (eluted with CH_2Cl_2-MeOH 95:5 v/v) successively gave (*E*)-*O*-geranylconiferyl alcohol (9*Z*, 12*Z*)-linoleate (45 mg), norchelerythrine (40 mg), and (*E*)-*O*-geranylconiferyl alcohol (120 mg). Similarly, flash chromatography performed on the MeOH extract (3 g), followed by column chromatographies on silica gel 20–45 μm, successively afforded magnoflorine (20 mg), hesperidin (220 mg), and methylbalfourodinium cation (12 mg).

Spectroscopic data

(E)-O-*Geranylconiferyl alcohol* (*9Z, 12Z*)-*linoleate* (**1**), UV (EtOH) λ_{max} (log ε) 267 (4.20), 292 (3.88) nm; IR (KBr) ν_{max} 3007, 2926, 2854, 1736, 1655, 1602, 1583, 1512, 1464, 1379, 1264, 1163, 1139, 1038, 999, 964, 855, 794, 725 cm^{-1}; 1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, *J* = 7 Hz 3 CH_3–18″), 1.25 (14H, m, CH_2–4″, 5″, 6″, 7″, 15″, 16″, 17″), 1.55 (3H, s, CH_3–9′), 1.61 (2H, m, CH_2–3″), 1.64 (3H, s, CH_3–10′), 1.68 (3H, s, CH_3–8′), 2.00 (6H, m, CH_2–4′, 8″, 14″), 2.07 (2H, m, CH_2–5′), 2.28 (2H, t, *J* = 7 Hz CH_2–2″), 2.75 (2H, m, CH_2–11″), 3.82 (3H, s, O-CH_3), 4.55 (2H, d, *J* = 7 Hz, CH_2–1′), 4.76 (2H, d, *J* = 7 Hz, CH_2–1), 5.04 (1H, t, *J* = 7 Hz, CH-6′), 5.31 (4H, m, CH-9″, 10″, 12″, 13″), 5.48 (1H, t, *J* = 7 Hz, CH-2′), 6.12 (1H, dt, *J* = 16 Hz, *J* = 7 Hz, H-2), 6.53, (1H, d, *J* = 16 Hz, H-3), 6.75 (1H, d, *J* = 8 Hz, H-8), 6.84 (1H, dd, *J* = 8 Hz, *J* = 1 Hz, H-9), 6.89 (1H, d, *J* = 1 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2 (C-18″), 16.7 (C-8′), 17.7 (C-9′), 22.9 (C-17″), 25.0 (C-3″), 25.7 (2C, C-10′, C-11″), 26.3 (C-5′), 27.3 (2C, C-8″, C-14″), 29.2 (2C, C-4″, C-5″), 29.4 (C-6″), 29.6 (C-15″), 29.8 (C-7″), 31.6 (C-16″), 34.3 (C-2″), 39.6 (C-4′), 55.8 (O-CH_3), 65.1 (C-1), 65.8 (C-1′), 109.1 (C-5), 113.0 (C-8), 119.9 (2C, C-9, C-2′), 121.2 (C-2), 123.9 (C-6′), 128.0 (C-12″), 128.1 (C-10″), 129.4 (C-4), 130.0 (C-9″), 130.1 (C-13″), 131.8 (C-7′), 134.3 (C-3), 140.6 (C-3′), 148.5 (C-7), 149.6 (C-6), 173.4 (C-1″); HR-ESI-MS found: 617.3968; 601.4241 (calcd for $[C_{38}H_{58}O_4 + K]^+$, 617.3972; calcd for $[C_{38}H_{58}O_4 + Na]^+$, 601.4233); ESI-MS *m/z* 617 $[M+K]^+$, 601 $[M+Na]^+$, 577, 301, 236.

Chemical correlation

Methanolysis of O-*geranylconiferyl alcohol* (*9Z, 12Z*)-linoleate (**1**): Sodium methanolate (5 mg) was added to a solution of **1** (20 mg) in anhydrous methanol. The mixture was stirred for 45 mn at 20 °C, neutralized with Amberlite® IRC50H^+ and filtered. The solvent was removed under reduced pressure. Column chromatography on silica gel 20–45 μm (solvent, cyclohexane-ethyl acetate 4:1 v/v) successively afforded methyl (9*Z*, 12*Z*)-linoleate (8 mg) identical with an authentic sample (TLC, IR, MS, ^1H- and ^{13}C-NMR) and (*E*)-*O*-geranylconiferyl alcohol (7 mg).

Arthur H. R., Hui W. H. and Ma C. N. (1956), Flavonoid glycosides from *Zanthoxylum* species and the occurrence of optically active hesperetin. J. Chem. Soc. 632–635.

Brader G., Wurz G., Greger H. and Hofer O. (1993), Novel prenylated 2-quinolinones from East Asian *Zanthoxylum* species. Liebigs Ann. Chem. 355–358.

Das Graças M. F., Da Silva F., Gottlieb O. R. and Ehrendorfer F. (1988), Chemosystematics of the Rutaceae : suggestions for a more natural taxonomy and evolutionary interpretation of the family. Pl. Syst. Evol. **161**, 97–134.

Doan Thi Mai H., Trinh Van-Dufat H., Michel S., Tillequin F., Bastien D. and Sévenet T. (2001), A new diprenyl coumarin and alkaloids from the bark of *Zanthoxylum dimorphophyllum* (Rutaceae). Z. Naturforsch. **56c**, 492–494.

Engler A. (1896), Rutaceae, In: Die natürlichen Pflanzenfamilien (A. Engler and K. Prantl, eds.) **III 4**, 95–201. Engelmann, Leipzig.

Engler A. (1931), Rutaceae, In: Die natürlichen Pflanzenfamilien (A. Engler and K. Prantl, eds.), 2te Auflage, **19a**, 187–359. Engelmann, Leipzig.

Guillaumin A. (1946), *Zanthoxylum*, In: Flore générale de l'Indochine (H. Lecomte and H. Humbert, eds.) **I**, Suppl. **1**, Fasc. **5**, 603–611. Muséum National d'Histoire Naturelle, Paris.

Guinaudeau H., Lebœuf M. and Cavé A. (1975), Aporphine alkaloids, Lloydia **38**, 275–338.

Gut B. J. (1966), Beiträge zur Morphologie des Gynoeceums der Blütenachse einiger Rutaceen. Bot. Jahrb. **85**, 10–247.

Ishii H. and Ishikawa T. (1976), Arnottianamide and isoarnottianamide: the structural establishment due to the conversion from the known benzo[*c*]phenanthridine alkaloids by the novel Baeyer-Villiger like oxidation of an immonium group. Tetrahedron Lett. 1203–1206.

Ishii H., Ishikawa T., Lu S.-T. and Chen I.-S. (1976), The chemical constituents of *Xanthoxylum cuspidatum* Champ. (= *Fagara cuspidata* Engl.). Yakugaku Zasshi **96**, 1458–1467.

Jen C.-M., Tsai I.-L., Horng D.-J. and Chen I. S. (1993), Isolation and structure determination of two new compounds from *Zanthoxylum integrifolium*. J. Nat. Prod. **56**, 2019–2021.

Krane B. D., Fagbule M. O., Shamma M. and Gözler B. (1984), The benzophenanthridine alkaloids. J. Nat. Prod. **47**, 1–43.

Lie Ken Jie M. S. F. and Mustafa J. (1997), High-resolution nuclear magnetic resonance spectroscopy – Applications to fatty acids and triacylglycerols. Lipids **32**, 1019–1034.

Moore J. A. (1936), Floral anatomy and phylogeny in the Rutaceae. New Phytol. **35**, 318–322.

Okamura N., Harguchi H., Hashimoto K. and Yagi A. (1994), Flavonoids in *Rosmarinus officinalis* leaves. Phytochemistry **37**, 1463–1466.

Pham Hoang H. (2000), Cây co Viêt Nam, **2**, 410. Nha Xuat Ban Tre, T. P. Hô Chi Minh.

Rapoport H. and Holden K. G. (1959), Isolation of alkaloids from *Balfourodendron riedelianum*. The structure of balfourodine. J. Am. Chem. Soc. **81**, 3738–3743.

Rideau M., Verchère C., Hibon P., Chénieux J.-C., Maupas, P. and Viel C. (1979), Alcaloïdes dihydrofuroquinoléiques de quelques Rutaceae: isolement, structure, propriétés biologiques. Phytochemistry **18**, 155–159.

Shibuya H., Takeda Y., Zhang R., Tong, R.-X. and Kitagawa I. (1992), On the constituents of the bark of *Fagara rhetza* (Rutaceae): alkaloids, phenylpropanoids and acid amide. Chem Pharm. Bull. **40**, 2325–230.

Slavik J. and Dolejs L. (1973), The constitution of escholinine and the identity of esholine with magnoflorine. Coll. Czech. Chem. Comm. **38**, 3514–3520.

Still W. C., Kahn M. and Mitra A. (1978), Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. **43**, 2923–2925.

Waterman P. G. and Grundon M. F. (1983), Chemistry and Chemical Taxonomy of the Rutales. Academic Press, London.