A New Diprenyl Coumarin and Alkaloids from the Bark of *Zanthoxylum dimorphophyllum* (Rutaceae)

Huong Doan Thi Mai[a], Hanh Trinh Van-Dufat[a], Sylvie Michel[a], François Tillequin[a,*], David Bastien[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 correspondence and reprint requests

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The alkaloids chelerythrine, norchelerythrine, oxyavicine, canthine-6-one, 4,5-dihydrocanthin-6-one, and γ-fagarine were isolated from *Zanthoxylum dimorphophyllum* bark, together with two coumarins, scoparone and dimoxylin. This latter is a novel compound whose structure was elucidated on the basis of its spectral data.

Introduction

The genus *Zanthoxylum* L. (Rutaceae) includes some 250 species of trees and shrubs, with a worldwide, but predominantly tropical distribution (Engler, 1896; Engler, 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). Surprisingly, none of the Vietnamese species have been so far studied from a chemical point of view. In a continuation of our studies on Rutaceous plants (Tillequin, 1997), we report here the isolation and structure determination of a novel coumarin, dimoxylin, together with the identification of a coumarin and six alkaloids, from the bark of *Zanthoxylum dimorphophyllum* Hemsl. (Hemsley, 1895) collected in Viêt Nam.

Results and Discussion

Eight secondary metabolites were isolated from the CH_2Cl_2 extract of *Zanthoxylum dimorphophyllum* bark. Three were identified as the benzophenanthridine alkaloids chelerythrine, norchelerythrine, and oxyavicine, widespread in the Rutaceae family (Krane *et al.*, 1984). Other known alkaloids included canthine-6-one (Ohmoto *et al.*, 1976), 4,5-dihydrocanthin-6-one (Rosenkranz and Schmid, 1968), and the furo[2,3-*b*]quinoline γ-fagarine (Narasimhan and Mali, 1974). The two remaining compounds were scoparone (6,7-dimethoxycoumarin) (Joseph-Nathan *et al.*, 1984) and a novel coumarin, dimoxylin.

Dimoxylin (**1**) was obtained as a white amorphous product. The empirical formula was determined by accurate mass measurement as $C_{20}H_{28}O_6$. The UV spectrum recorded in MeOH was suggestive of a 7-oxygenated coumarin. The IR spectrum showed characteristic bands at 3415 and 1712 cm^{-1} accounting for alcoholic hydroxyl groups and for the pyrone-carbonyl, respectively. In the aromatic region, the ^1H NMR spectrum displayed a pair of doublets (J = 9.5 Hz) at 6.21 and 7.58 ppm typical for a coumarin unsubstituted in the pyrone ring, whereas a singlet at 7.28 ppm was consistent with the presence of three substituents in the aromatic ring. At higher field, typical signals accounted for one aromatic methoxyl and two different *C*-prenyl derived substituents, a 2,3-dihydroxy-3-methylbutyl side chain, and a 3-hydroxy-3-methylbutyl group. The assignment of the methoxyl and prenyl moieties was carried out using multi-impulsional HETCOR and COLOC experiments. Of particular interest were the following three bond COLOC connectivities: i) H-1′a at 2.57 ppm and H-1′b at 2.90 ppm to C-5 at 127.7 ppm ii) H-5 at 7.28 ppm to C-4 at 143.9 ppm,

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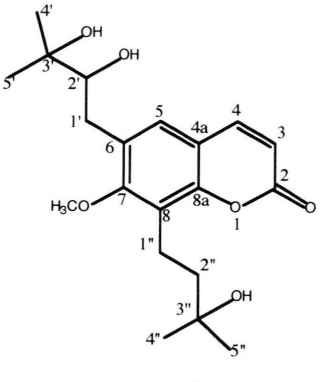
C-7 at 159.8 ppm, C-8a at 151.7 ppm, and C-1′ at 32.3 ppm. These data permitted to locate unambiguously the 2,3-dihydroxy-3-methylbutyl substituent at C-6, and hence the 3-hydroxy-3-methylbutyl chain at C-8. Therefore, the structure of dimoxylin can be depicted as **1**. The absolute configuration of the chiral center at C-2′ could not be determined, due to the small amount of product isolated.



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The isolation of coumarins together with that of alkaloids belonging to the benzophenanthridine, canthinone, and furoquinoline series indicates a lack of high skeletal specialization in the biosynthesis of *Zanthoxylum dimorphophyllum* secondary metabolites. From a chemotaxonomic point of view, it 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 rotation was obtained on a Perkin Elmer 241 polarimeter. Specific rotation ($[\alpha]_D$) is reported in deg/dm. UV spectrum (λ_{max} in nm) was recorded in spectroscopically grade MeOH on a PU 8700 Philips spectophotometer. IR spectrum (ν_{max} in cm^{-1}) was obtained from potassium bromide pellet on a Nicolet FT-IR 510 instrument. ^{1}H-NMR (δ [ppm], *J* [Hz]) and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, using a Bruker AC-300 spectrometer. Multi-impusional 2D NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{13}C-^{1}H HETCOR, and ^{13}C-^{1}H COLOC) were performed using standard Bruker microprograms. High resolution mass spectrum (HR-MS) and Fast Atom Bombardment (FAB-MS) were recorded on a Micromass ZAB 2 – SEQ spectrometer. Mass spectra was recorded with a Nermag R 10–10C spectrometer, using desorption-chemical ionization (DCI-MS; reagent gas: NH3) technique. 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 mbars (Still *et al.*, 1978). Microanalyses were in agreement with calculated values ±0.4%.

Plant Material

Bark of *Zanthoxylum dimorphophyllum* Hemsl. was collected at Pà Cò, Mai Châu (Hoà Bình, Viêt Nam), on 4 March 1997. A Voucher sample (VN 227) is kept in the herbarium of the Institute of Ecology of the National Center for Science and Technology in Hà Nôi, Viêt Nam.

Extraction and Isolation

Dried, pulverized bark of *Zanthoxylum dimorphophyllum* (1 kg) was extracted with CH_2Cl_2 (4 × 2 l) at room temperature. The solvent was removed under reduced pressure to give a crude extract (15.3 g). An aliquot (2.5 g) was subjected to flash column chromatography on silica gel, using a CH_2Cl_2-MeOH gradient of increasing polarity to yield 12 fractions. Further column chromatographies on silica gel 20–45 μm, performed on fractions 4 to 10, successively gave chelerythrine (34 mg), norchelerythrine (40 mg), scoparone (20 mg), γ-fagarine (28 mg), oxyavicine (22 mg), canthine-6-one (120 mg), dimoxylin (65 mg), and 4,5-dihydrocanthin-6-one (12 mg).

Spectroscopic data

Dimoxylin (**1**), $[\alpha]^{20}_{D}$ +23.5 (1 g/100 ml, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 217 (4.39), 252 (3.68), 294 (4.12), 325 (3.98) nm; IR (KBr) ν_{max} 3415, 3055, 2972, 2936, 1712, 1606, 1567, 1470, 1396, 1134, 1051, 907, 828, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (9H, s, 3 C-CH_3), 1.30 (3H, s, C-CH_3), 1.68 (2H, m, CH_2-2″), 2.57 (1H, dd, *J* = 14 Hz, *J* = 9 Hz, H-1′a), 2.76 (2H, m, CH2-1″), 2.90 (1H, dd, *J* = 14 Hz, *J* = 1 Hz, H-1′b), 3.10 (3H, br.

s, D_2O exch., 3 OH), 3.60 (1H, dd, $J = 9$ Hz, $J = 1$ Hz, H-2′), 3.78 (3H, s, O-CH_3), 6.21 (1H, d, $J = 9.5$ Hz, H-3), 7.28 (1H, s, H-5), 7.58 (1H, d, $J = 9.5$ Hz, H-4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.6 (C-1″), 23.7 (C-CH_3), 26.0 (C-CH_3), 28.8 (C-CH_3), 29.0 (C-CH_3), 32.3 (C-1′), 42.9 (C-2″), 61.8 (O-CH_3), 70.5 (C-3″), 72.8 (C-3′), 78.2 (C-2′), 114.2 (C-3), 115.2 (C-4a), 124.1 (C-8), 127.7 (C-5), 130.0 (C-6), 143.9 (C-4), 151.7 (C-8a), 159.8 (C-7), 161.4 (C-2); HR-FAB-MS found: 387.1768 (calcd for $[C_{20}H_{28}O_6 + Na]^+$, 387.1783); DCI-MS *m/z* 382 $[M+NH_4]^+$, 365 [MH]+, 347.

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