Alkamides from the Leaves of *Zanthoxylum syncarpum*

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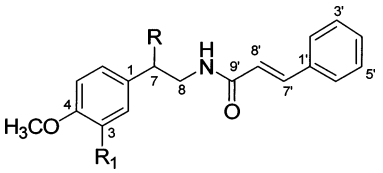
Three alkamides (**1**−**3**) were isolated from the leaves of *Zanthoxylum syncarpum.* The structures of the new compounds **1** and **2** were established by spectroscopic data and chemical conversion, and by the X-ray crystallography of **1**. Compound **3**, the racemic form of the known compound syncarpamide, showed moderate antiplasmodial activity, with IC_{50} values of 4.2 and 6.1 μM against *Plasmodium falciparum* D6 clone and W2 clone, respectively.

Zanthoxylum, commonly called "prickly ash", is the largest genus in the family Rutaceae and comprises about 200 species of trees and shrubs, with a worldwide, but predominantly tropical, distribution.[1,2] *Zanthoxylum* species are reported to have many medicinal properties,[3] and phytochemical studies on the genus have shown it to be a rich source of coumarins, lignans, and alkaloids, with febrifuge, sudorific, and diuretic properties.[4−16]

Zanthoxylum syncarpum Tul. is an evergreen tree occurring in both North and South America.[13] Although there are several reports on the chemical constituents of various *Zanthoxylum* species,[5−11] there are only four previous reports on *Z. syncarpum*.[12−15] In view of the potential biological properties of *Zanthoxylum* species, we have investigated a methanol extract of the leaves of *Z. syncarpum*, since it exhibited potent in vitro bioactivity (81%) against a chloroquine-sensitive (D6, Sierra Leone) strain of *Plasmodium falciparum*, and have isolated two new alkamides (**1** and **2**) and the racemic form of syncarpamide (**3**),[12] which are the subject of the present paper.



1 R = OH; R_1 = OMe
2 R = OAc; R_1 = OMe
3 R = cinnamoyl; R_1 = OMe

The leaves of *Z. syncarpum* were collected in Lara State, Venezuela, and were air-dried, ground, and exhaustively extracted with methanol at room temperature, with the extract concentrated under reduced pressure. The concentrate (80 g) was chromatographed over a silica gel column, and further workup as described in the Experimental Section resulted in the isolation and purification of three compounds (**1**−**3**).

Compound **1** was obtained as white crystalline aggregates from EtOAc−MeOH (mp 138−139 °C) and gave a positive test with Dragendorff's reagent. It showed a protonated molecular ion $[M + H]^+$ peak at *m/z* 328.1546 (calcd 328.1543) in the HRESIMS, and the resulting molecular formula was determined to be $C_{19}H_{21}NO_4$, representing 10 degrees of unsaturation. The IR bands at 3337, 1713, 1656, 1615, and 1516 cm^{-1}, respectively, indicated the presence of hydroxyl, carbonyl, olefinic, and amide functionalities in **1**. The ^1H and ^{13}C NMR spectra of **1** gave signals including five quaternary carbons, 11 methines, one methylene, and two methyl carbons. Two olefinic signals at δ 7.57 (141.8, C-7′) and 6.39 (120.4, C-8′), each integrating for one proton with a J value of 15.6 Hz, indicated that they were *trans* coupled, and these assignments were confirmed using the $^1H-^1H$ COSY spectrum. The corresponding olefinic carbons were assigned by their HMQC correlations. The proton signals at δ 6.92 (1H, d, J = 1.2 Hz), 6.85 (1H, dd, J = 1.6, 8.0), and 6.75 (1H, d, J = 8.4 Hz), and signals at δ 7.40 (2H, dd, J = 1.6, 6.8 Hz) and 7.30 (3H, m), indicated the presence of 1,3,4- and monosubstituted benzene rings in **1**. Two singlets, each integrating for three protons at δ 3.80 and 3.79, suggested the presence of two methoxy groups, which were placed at C-3 and C-4, on the basis of HMBC correlations (Table 1). A deshielded secondary carbon at δ 48.1 and a methine carbon at δ 73.6 supported the presence of nitrogen- and oxygen-bearing carbons in **1**. These spectroscopic features revealed that compound **1** has the general structural features of aegeline.[16,17] The NMR data of both these compounds showed similar patterns, except for the presence of an additional methoxy group in **1**, which was placed at C-3 on the basis of HMBC correlations. Thus, compound **1** was characterized as a methoxy derivative of aegleine, and data from the $^1H-^1H$-COSY, HMQC, and HMBC NMR spectra (Table 1) provided additional support to justify the gross structure shown for **1**. Compound **1** (3-methoxyaegeline) did not show any optical rotation, indicating that it was obtained as a racemic mixture. Recrystallization from EtOAc−MeOH afforded racemic crystals, and unequivocal evidence for the assigned structure was obtained from an X-ray diffraction analysis (Figure 1). Interestingly, the crystals are kryptoracemic; that is, the space group does not contain symmetry elements that invert the handedness, but the asymmetric unit is a pair of enantiomers. Such crystallization behavior from a racemic solution is uncommon.

Compound **2** was obtained as a light yellow, amorphous powder and showed a molecular ion peak $[M + Na]^+$ at *m/z* 392.1462 (calcd for $C_{21}H_{23}NO_5Na$, 392.1474) in the

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Table 1. ^{13}C, ^{1}H, and HMBC NMR Spectroscopic Data for Compounds **1** and **2** ($CDCl_3$)[a]

	1			**2**		
position	δ_C	δ_H	HMBC (H to C)	δ_C	δ_H	HMBC (H to C)
1	134.8 s			130.2		
2	109.3 d	6.92 d (1.2)	3,6,7	109.8 d	6.94 d (1.2)	6,7,3,4
3	149.3 s			149.2 s		
4	148.8 s			149.1 s		
5	111.4 d	6.75 d (8.4)	3	111.2 d	6.86 d (8.4)	1,3,4
6	118.3 d	6.85 dd (1.6, 8.0)	1,2,4,7	119.1 d	6.97 dd (1.6, 8.0)	2
7	73.6 d	4.82 dd (3.2, 8.4)		74.7 d	5.94 dd (4.8, 8.0)	1,2,6,7
8	48.1 t	3.73 H_a, dd (3.2, 4.8) 3.45 H_b, m	7,9′	44.5 t	3.75 dd (3.2, 4.4)	
9				170.2 s		
10				23.32 q	2.03 s	10
1′	134.8 s			134.2 s		
2′	128.1 d	7.40 dd (1.6, 6.8)	1′,3′,7′	128.2 d	7.54 t (3.6, 6.4)	3′
3′	129.0 d	7.30 m		128.9 d	7.41 m	
4′	130.1 d	7.30 m		130.6 d	7.41 m	
5′	129.0 d	7.30 m		128.9 d	7.41 t (3.6, 6.4)	5′
6′	128.1 d	7.40 dd (1.6, 6.8)	1′,5′,7′	128.2 d	7.54 m	8′,9′
7′	141.8 d	7.57 d (15.6)	2′,5′,9′	145.8 d	7.73 d (16.0)	1′,7′,9′
8′	120.4 d	6.39 d (15.6)	1′,9′	117.6 d	6.50 d (16.0)	
9′	167.6 s			166.5 s		
OCH_3-3	56.1 q	3.80 s	3,4	55.9 q	3.92 s	3,4
OCH_3-4	56.1 q	3.79 s	3,4	55.9 q	3.89 s	3,4
NH-8		6.63 br s			5.80 br s	7

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, carbon multiplicities were determined by DEPT experiments. s = C, d = CH, t = CH_2. Figures in parentheses denote *J* values (Hz).

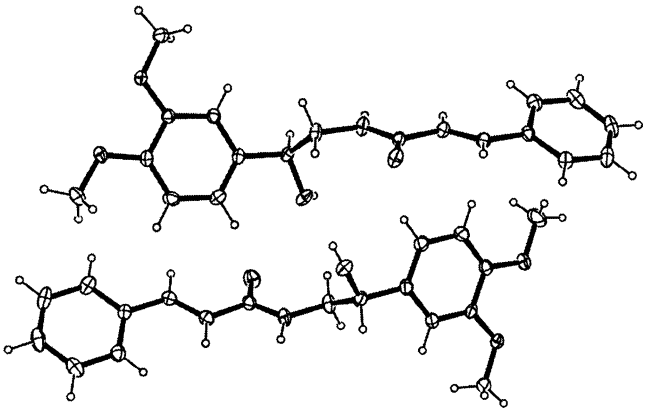

Figure 1. ORTEP diagram of two independent, enantiomeric molecules of **1**.

positive HRESIMS, corresponding to the molecular formula $C_{21}H_{23}NO_5$. IR absorption bands at 3368 and 3281 cm^{-1} indicated the presence of a secondary amide group, and a band at 1713 cm^{-1} suggested the occurrence of an ester group, which were supported by observations in the ^{13}C NMR spectrum of an amide carbonyl signal at δ 170.2, an ester carbonyl signal at δ 166.5, an oxygen-attached carbon signal at δ 74.7, and a nitrogen-attached carbon signal at δ 44.5 (Table 1). Two olefinic proton doublets ($J = 16.0$ Hz), each integrating for one proton at δ 7.73 (1H, d, $J = 16.0$, H-7′) and 6.50 (1H, d, $J = 16.0$ Hz, H-8′), were attributable to the presence of a *trans*-olefinic system in **2**. The ^{1}H NMR spectrum also showed the presence of 1,3,4-trisubstituted and monosubstituted benzene rings and two methoxy functionalities in **2**. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of compound **1** (Table 1) indicated that **2** is an acetyl analogue of **1**, and the molecular formula of **2** supported the presence of this additional group. This group was located at the C-7 position, which was suggested by the HMBC correlations (Table 1). From these findings, compound **2** was characterized as 3-methoxy-7-acetylaegeline (**2**). Unequivocal evidence for this structure was obtained by acetylation of **1**, which provided an acetyl derivative with physicochemical properties closely comparable to **2**.

The identification of **3** was based on analogies made with **1**. Thus, comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **1** and **3** revealed identical spin systems except for an additional cinnamoyl group. Literature data indicated that **3** has the same structural features as syncarpamide,[12] having similar physical properties with the exception of its optical rotation value. Syncarpamide showed $[\alpha]_D^{25}$ +12.5° (*c* 0.08, $CHCl_3$), while compound **3** gave $[\alpha]_D^{25}$ 0° (*c* 0.13), using the same solvent. These data supported the identification of compound **3** as 3-methoxy-7-cinnamoylaegeline (syncarpamide), which we have recently reported from the stems of the same plant.[12] Compound **3** is the racemic form of this substance.

As the MeOH crude extract of *Z. syncarpum* showed potent in vitro bioactivity against a chloroquine-sensitive (D6, Sierra Leone) strain of *P. falciparum*, the antimalarial activity of the isolated compounds (**1**−**3**) was evaluated against chloroquine-sensitive (D6, Sierra Leone) and -resistant (W2, Indochina) strains of *P. falciparum*. Compounds **1** and **2** were inactive, whereas compound **3** showed moderate activity, with IC_{50} values of 4.2 and 6.1 μM against *P. falciparum* D6 clone, Sierra Leone, and *P. falciparum* W2 clone, Indochina, respectively. Artemisinin and chloroquine showed IC_{50} values of 0.04 and 0.05 μM against *P. falciparum* D6 clone and IC_{50} values of 0.03 and 0.5 μM against *P. falciparum* W2 clone, respectively. Cytotoxicity was evaluated at an IC_{50} of 4.7 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-310 digital polarimeter. UV and IR spectra were obtained using a Perkin-Elmer Lambda 3B UV/vis spectrophotometer and an AATI Mattson Genesis Series FT-IR spectrometer, respectively. The ^{1}H and ^{13}C NMR spectra as well as 2D NMR spectra (COSY, HMQC, HMBC) were recorded in $CDCl_3$ using Bruker, DRX NMR spectrometers operating at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C using the solvent peak as internal standard. The HRMS were measured using a Bioapex FTESI-MS, with

electrospray ionization. A Chromatotron 8924 (Harrison Research, Palo Alto, CA) with silica gel 60 PF 254 (1 mm) plates was used. TLC analysis was carried out on precoated silica gel G_{254} or aluminum oxide ALOX-100 UV_{254} 500 μm plates with visualization by 5% H_2SO_4 in EtOH and heating with Dragendorff's reagent. Single-crystal-X-ray diffraction measurements were made on a Nonius Kappa CCD diffractometer with Mo Kα radiation.

Plant Material. Leaves of *Z. syncarpum* Tul. were identified and collected in Lara State, Venezuela, by one of the authors (C.L.B.) in February 2002. A voucher specimen, BUR 230202 (1), is deposited at the private herbarium of this author (518 Audubon, Oxford, MS 38655, e-mail: in_the_200@mail2farm.com).

Extraction and Isolation. The air-dried and powdered leaves *Z. syncarpum* (412 g) were extracted with MeOH at room temperature. The extract was concentrated under reduced pressure to yield 80 g of residue. The methanol extract (80 g) was subjected to silica gel vacuum liquid chromatography (750 g of silica gel, 5.25 μm) and eluted beginning with *n*-hexane (100%), *n*-hexane−chloroform (1:1), chloroform (100%), and finally ethyl acetate−methanol (10%−100%). Altogether, 25 major fractions (250 mL each) were collected, and the elution was monitored by TLC.

Fractions 9−17 (6.5 g) were combined and passed over another silica gel column (250 g), eluted with chloroform−ethyl acetate (25%−100%), followed by step gradient elution with ethyl acetate−methanol (10%−100%). In total, 20 fractions were collected. 3-Methoxyaegeline (**1**, 30 mg), from fraction 8, was obtained by recrystallization of an ethyl acetate−methanol mixture at room temperature. Fraction 17 (3.5 g) was further chromatographed over an alumina column (38 × 39 cm; 350 g basic alumina, Sigma-Aldrich) and eluted with chloroform (100%) and chloroform−methanol (2% to 25%). A total of 17 fractions were collected and were divided into three groups: group A (1), group B (2−4), and group C (5−17). Group B (fractions 2−4, 0.9 g) was further fractionated by silica gel chromatography with chloroform−methanol (0.5%−20%) to afford 3-methoxy-7-acetylaegeline (**2**, 28 mg) and 3-methoxy-7-cinnamoylaegeline (**3**, 54 mg). Group C (fractions 5−17, 1.4 g) was further purified using the Chromatotron (hexane−$CHCl_3$−MeOH, 1:92.5:6.5), followed by recrystallization in ethyl acetate−methanol, to obtain an additional quantity of 3-methoxyaegeline (**1**, 355 mg).

3-Methoxyaegeline (1): white crystals (EtOAc−MeOH), mp 138−139 °C, $[\alpha]_D^{25}$ 0° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 218 (4.37), 225 (4.57), 278 (4.65) nm; IR (KBr) ν_{max} 3337, 2920, 2851, 2358, 2339, 1713, 1656, 1615, 1540, 1516, 1451, 1261, 1231, 1027, 977 cm^{-1}; NMR data, see Table 1; HRMS *m/z* 328.1546 (calcd for $C_{19}H_{21}NO_4$, $[M + H]^+$, 328.1543).

X-ray Crystal Structure Determination of 1. A suitable crystal of **1** was obtained by slow crystallization from EtOAc−MeOH (1:1) at room temperature for 3 days. Crystal data: $C_{19}H_{21}NO_4$, monoclinic space group $P2_1$, a = 11.684(3) Å, b = 9.293(2) Å, c = 15.710(5) Å, β = 102.586(10)°, V = 1664.8(8) Å^3, Z = 4, R = 0.048 ($F^2 > 2\sigma$), R_w = 0.103 (all F^2) for 5335 unique data having $2\theta < 61°$ and 450 refined parameters. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 274925. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

3-Methoxy-7-acetylaegeline (2): light yellow, amorphous powder; $[\alpha]_D^{25}$ 0° (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (4.82), 252 (4.67), 282 (4.98) nm; IR (KBr) ν_{max} 3368, 3281, 2953, 1713, 1659, 1517, 1450, 1263, 1162, 1026, 975, 766 cm^{-1}; NMR data, see Table 1; HRMS *m/z* 392.1462 (calcd for $C_{21}H_{23}NO_5$, $[M + Na]^+$, 392.1474).

Conversion of Compound 1 to 2. Treatment of **1** (10 mg) with acetic anhydride (250 μL) and pyridine (250 μL) at room temperature for 4 days and subsequent workup gave **2** (8 mg), with ^1H NMR and ^{13}C NMR data identical to natural compound **2**.

3-Methoxy-7-cinnamoylaegeline (3): light yellow, amorphous powder; $[\alpha]_D^{25}$ 0° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} 242 (4.83), 252 (4.69), 278 (4.94) nm; IR (KBr) ν_{max} 3472, 2961, 2923, 1713, 1604, 1449, 1268, 757 cm^{-1}; NMR data, see Table 1; HRMS *m/z* 480.1821 (calcd for $C_{28}H_{27}NO_5$, $[M + Na]^+$, 480.1787).

Evaluation of in Vitro Antiparasitic and Anti-infective Activities. Antimalarial activity of the compounds was determined in vitro on chloroquine-sensitive (D6, Sierra Leone) and -resistant (W2, Indochina) strains of *P. falciparum*. This 96-well microplate assay is based on evaluation of the effect of the compounds on the growth of asynchronous cultures of *P. falciparum*, determined by the evaluation of parasite lactate dehydrogenase (pLDH) activity.[17] The appropriate dilutions of the compounds were prepared in RPMI 1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set up in clear flat-bottomed 96-well plates. The plates were placed in a modular incubation chamber, flushed with a gaseous mixture of 90% N_2, 5% CO_2, and 5% O_2, and incubated at 37 °C for 48 h. Growth of the parasite in each well was determined by the pLDH assay using Malstat reagent. The medium and RBC controls were included on each plate. The standard antimalarial agents, chloroquine and artemisinin, were used as the positive controls, while DMSO was tested as the negative control.

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