Syncarpamide, a New Antiplasmodial (+)-Norepinephrine Derivative from *Zanthoxylum syncarpum*

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A new (+)-norepinephrine derivative, syncarpamide (**1**), along with a known coumarin, (+)-*S*-marmesin (**2**), and one known alkaloid, decarine (**3**), have been isolated from the stem of *Zanthoxylum syncarpum*. The structure of compound **1** was elucidated on the basis of 1D and 2D NMR, MS, IR, optical rotation, and CD analyses. Its absolute stereochemistry was elucidated by synthesis of its enantiomer and subsequent comparison of CD data. Characterizations of compounds **2** and **3** were based on spectral analysis and comparison with reported data. Compounds **1** and **3** showed antiplasmodial activity, with IC_{50} values of 2.04 and 1.44 μM against *Plasmodium falciparum* D_6 clone and 3.06 and 0.88 μM against *P. falciparum* W_2 clone, respectively. Compound **3** showed cytotoxicity at 56.42 μM, whereas compound **1** was not cytotoxic at 10.42 μM. Compound **1** was tested for hypotensive activity, but no activity was observed. Compound **2** showed no antiplasmodial or antimicrobial activities.

The genus *Zanthoxylum* (Rutaceae) comprises some 200 species distributed worldwide. Plants of this genus exhibit a variety of biologically active secondary metabolites including alkaloids, lignans, and coumarins with febrifuge, sudorific, and diuretic properties.[1] The bark of *Zanthoxylum integrifoliolum* (Merr.) Merr. (Rutaceae) has been utilized in traditional medicine by Ya-Mei aborigines in Lanyu Island in Taiwan as a remedy for snakebite[2] and dyspepsia and as an aromatic tonic in fever.[3] In Sri Lanka, the genus *Zanthoxylum* is represented by three species, viz., *Z. caudatum*, *Z. rhetza*, and *Z. tetraspermum*.[4] Of these, *Z. tetraspermum* (*Sinhala*-katukeena) is employed in folklore medicine for the treatment of rheumatism and some forms of diarrhea.[5] Pure compounds with antimicrobial activity have been obtained from the root bark of *Z. gilletii*.[6] The bark is pungent, and sticks prepared from it are used for preventing toothache.[7] The stem bark of *Z. liebmanniaum* (Engelm.) P. Wilson is used in Mexican traditional medicine for the treatment of stomach aches, amebiasis, and intestinal parasites and as a local anesthetic agent.[8]

Z. syncarpum Tul. is an evergreen tree occurring in both North and South America.[9] Although there are several reports on the chemical constituents of different species of *Zanthoxylum*,[3−8] only two papers dealt with the constituents of *Z. syncarpum*.[10,11] These phytochemical studies revealed the presence of two alkaloids, skimmianine and *cis*-*N*-methylcanadine, two coumarins, xanthotoxin and isopimpinellin, a diterpene, centipedic acid, the flavonoid ternatin, the triterpene lupeol, β-sitosterol glucoside,[10] and an essential oil.[11]

This paper deals with the isolation and characterization of a new alkaloid (**1**) and also the first report of the presence of **2** and **3** in *Z. syncarpum.* The isolation of these types of alkaloids and coumarins is of chemotaxonomic importance, as structurally related alkaloids have been reported earlier from *Fagara* and *Zanthoxylum* species.[12,13]

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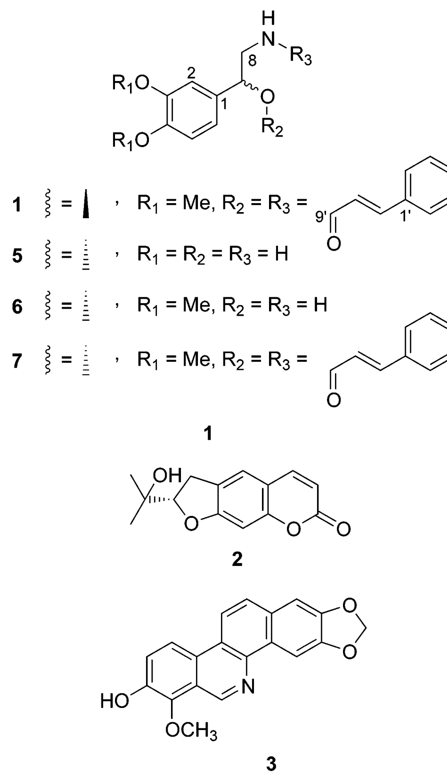
Stems of *Z. syncarpum* were air-dried, ground, and sieved through a 1.0 mm mesh. An ethanolic extract of this material was carefully chromatographed over a silica gel column. The collected fractions were further purified by repeated chromatography over open silica gel columns, reversed-phase HPLC, and crystallization to afford compounds **1**, **2**, and **3**.



Syncarpamide (**1**) was obtained as light brown solid, $[\alpha]_D$ +12.5°, which gave a positive test with Dragendorff's reagent. The molecular formula was established as $C_{28}H_{27}NO_5$ by HRESIMS with 16 degrees of unsaturation. Analysis of ^1H NMR data, supported by $^1H-^1H$ COSY spectra,

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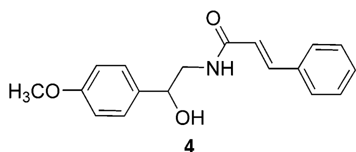
revealed the presence of two pairs of *trans* olefinic systems at δ 6.38 (1H, d, $J = 15.5$ Hz), 7.63 (1H, d, $J = 15.5$ Hz), and δ 6.50 (1H, d, $J = 16.0$ Hz), 7.72 (1H, d, $J = 16.0$ Hz). The IR absorption band at 1713 cm^{-1} indicated the presence of a carbonyl group, and corresponding carbon peaks at δ 166.0 and 166.5 in the ^{13}C NMR spectra were reminiscent of an amide and an ester bond in **1**. In the HMBC spectra, the first pair of *trans* olefinic protons showed cross-peaks with the carbonyl carbon at δ 166.0 and the second pair with the carbon at δ 166.5. Analysis of ^1H and ^{13}C NMR data also revealed that **1** contained a 1,3,4-trisubstituted and two monosubstituted benzene rings. HMBC connectivity linked each pair of olefinic protons to a 1-substituted benzene ring. Two *O*-methyl singlets at δ 3.87 and 3.90 of the 1, 3, 4-trisubstituted benzene rings were observed in the ^1H NMR spectrum. Cross-peaks in the HMBC spectrum indicated that these methoxy groups are attached to the aromatic carbons at δ 149.1 and 149.2, respectively. One more spin system at δ_H 6.00 (1H, t, $J = 6.4$ Hz), δ_C 74.7, and δ_H 3.88 (2H, overlapped with methoxy signals), δ_C 44.7 was observed. The singlet aromatic resonance of the 1,3,4-trisubstituted benzene ring at δ_H 6.96 (δ_C 109.8) showed a cross-peak in the HMBC spectra to the oxymethine signal at δ_H 6.00 of the aliphatic spin system. The characteristic deshielded secondary carbon at δ_C 44.7 of the latter spin system indicated that it was connected to the nitrogen atom. A deshielded oxymethine proton resonance at δ_H 6.00 also indicated *O*-cinnamoylation at this position. Thus the structure of syncarpamide was elucidated as shown in **1**. It bears some resemblance to the naturally occurring aegline **4**, which was obtained in racemic form.[13,14]



The positive specific rotation of syncarpamide (**1**) indicated that it was probably a derivative of (+)-*S*-norepinephrine. To confirm the absolute configuration of (**1**), we opted to derivatize commercially available (−)-*R*-norepinephrine (**5**) with a view to comparing its chiroptical properties with those of syncarpamide (**1**). Thus, methylation of the sparingly soluble (−)-*R*-norepinephrine (**5**) with ethereal diazomethane in a large excess of MeOH afforded (−)-*R*-3,4-di-*O*-methylnorepinephrine (**6**). Treatment with cinnamoyl chloride (2.1 equiv) in pyridine gave the *N,O*-dicinnamoyl derivative (**7**). Its CD spectrum exhibited sequential high-amplitude positive and negative Cotton effects in the 290−300 and 260−270 nm regions for the n→p* and π→p* transition of the α,β-unsaturated carbonyl chromophores, respectively. The CD spectrum of syncarpamide (**1**) showed a mirror image type relationship to that of **7** (Figure 1), hence unequivocally confirming the *S*-absolute configuration of the new naturally occurring (+)-norepinephrine derivative (**1**).

Compound **2** was identified as (+)-*S*-marmesin by comparison of IR, HREIMS, and 1D and 2D NMR spectral data with published values.[15,16]

Compound **3** was identified as decarine by comparison of IR, HREIMS, and 1D and 2D NMR spectral data with published values.[17,18] Decarine was also isolated from *Z. decaryi*[18] and *Z. viride*.[19]

Syncarpamide (**1**) and decarine (**3**) showed strong in vitro antiplasmodial activity[21] against *Plasmodium falciparum*, having IC_{50} values of 2.04 and 1.44 μM against the D_6 strain and 3.06 and 0.88 μM against the W_2 strain, respectively.[22,23] These compounds showed no cytotoxicity, except decarine at high concentration (56.42 μM). Decarine isolated from *Z. tetraspermum* and *Z. coudatum* showed significant antibacterial activity.[20] Marmesin (**2**) was not active in either the antimicrobial or antiplasmodial screen.

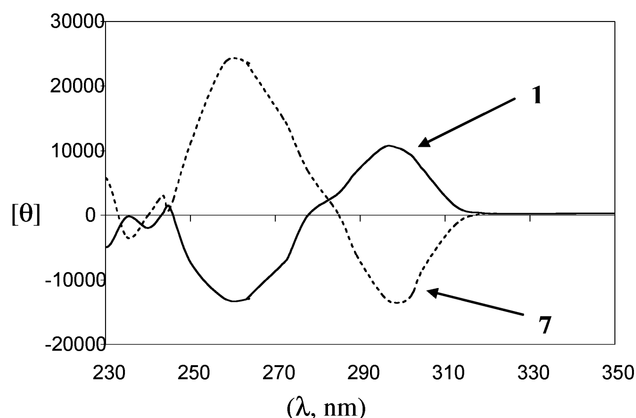


Figure 1. CD spectra of syncarpamide **1** and its (−)-*R*-norepinephrine enantiomer **7**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a RUDLPH Research Autopol IV automatic polarimeter. UV spectra were recorded using a Hewlett-Packard 8453 instrument. IR spectra were obtained using an AATI Mattson Genesis Series FTIR. ^1H and ^{13}C NMR spectra as well as 2D spectra (COSY, HMQC, and HMBC) were recorded on a Bruker DRX 400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C using the solvent peaks as internal references. EIMS was acquired on a GC/MS: Hewlett-Packard 5890A gas chromatography (GC) interface with 5970A selection detector (MSD). The GC/MS system was fitted with a DB-1 column (15 m × 0.25 mm, and 0.25 μm film thickness, J & W Scientific, Folsom, CA), and HRESIMS and ESIMS data were acquired on a Bruker BioAPEX 30es mass spectrometer. TLC was performed on Merck TLC plates precoated with Si_{60} F_{254} or Si_{60} RP18 F_{254} with visualization by spraying with 5% H_2SO_4 in EtOH and heating or by Dragendorff's reagent. HPLC was carried out on a Waters Millennium system with a 996 photodiode array detector. CD spectra were recorded in $CHCl_3$ (ca. 0.1 mg/mL $CHCl_3$) on a JASCO J-715 spectrometer; scan parameters: bandwidth (2.0 nm), sensitivity (10 mdeg), response (4 s), scan speed (50 nm/min), step resolution (0.1 nm).

Plant Material. Specimens of *Z. syncarpum* were identified and collected in Lara State, Venezuela, by Dr. Charles L. Burandt in February 2002. Dried and ground stems were used for the isolation. A voucher specimen, BUR230202 (2), is deposited at the private herbarium of Dr. Burandt.

Extraction and Bioassay. The powdered, air-dried stems (1.2 kg) were exhaustively extracted with MeOH (3 × 2 L). The combined MeOH extracts were evaporated, and the residue (63 g, 5.3% yield of dry plant weight) was chromatographed on a silica gel column (380 × 78 mm, 540 g of silica 32−63 μm, 100:0:0−0:0:100 hexanes−EtOAc−MeOH step gradient) to give eight fractions, C_1-F_1, C_1-F_2, C_1-F_3, C_1-F_4, C_1-F_5, C_1-F_6, C_1-F_7, and C_1-F_8 (1 L each). Combined fractions C_1-F_2 to C_1-F_4 (6.23 g) were further chromatographed on silica gel (280 × 35 mm column, 160 g of silica 32−63 μm, eluted with 100:0−0:100 $CHCl_3$−MeOH step gradient) to give 10 fractions. Fraction C_2-F_5 (1.83 g) was further chromatographed on silica gel (150 × 35 mm column, 60 g of silica 32−63 μm, eluted with 98:2:0−0:0:100 hexane−EtOAc−MeOH step gradient) to give fractions F_5-1, F_5-2, F_5-3, F_5-4, F_5-5, and F_5-6. Fraction F_5-3 was crystallized from an EtOAc−hexane mixture

to afford compound **3** (44.0 mg). Fraction F_5-4 was crystallized from a MeOH−EtOAc mixture to give compound **2** (8.5 mg), and the mother liquid was subjected to reversed-phase HPLC (Prodigy ODS-3, 5 μm, 21.2 × 250 mm column, 70:30 (v/v) MeOH−H_2O, 14 mL min^{-1}, photodiode-array detection monitored at 220 nm) separation to afford compound **1** (5.9 mg).

L-Norepinephrine and cinnamoyl chloride were purchased from Acros Organics, NJ. Diazald, 99% (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) was purchased from Aldrich Chemical Co. Inc.

Cytotoxicity Assay. In Vitro Cytotoxic Activity. The level of toxicity of each sample was determined by measuring the effect on a fibroblast cell line from African green monkey kidney (VERO, nontransformed). For initial (primary) evaluation, extracts and fractions were screened at a single concentration (100 μg/mL). Follow-up secondary assays were conducted at three concentrations (4.76, 1.58, and 0.52 μg/mL), using a culture-treated 96-well microplate 16. For secondary assay, IC_{50} values were determined from logarithmic graphs of growth inhibition values. The cytotoxic agents doxorubicin and 5-fluorouracil were used as positive controls, while DMSO was used as the negative (vehicle) control.

Antimicrobial Assay. Preliminary antimicrobial activities of the crude exctracts/fractions and the IC_{50}/MIC values of compounds were determined using a modified 96-well microplate assay protocol.[24] The test organisms used were ATCC strain of *Candida albicans* B311 (#90028), *Cryptococcus neoformans* (#90113), *Stephylococcus aureus* (#6535), and *S. aureus* (# 33591). Amphotericin B and rifampin were used as positive controls, with DMSO as a negative control.

Syncarpamide (1): light brown solid, $[\alpha]_D^{25}$ +12.50° (*c* 0.08, $CHCl_3$); IR (film) ν_{max} 3472, 2961, 2923, 1713, 1604, 1449, 1268, 757 cm^{-1}; UV λ_{max} (MeOH) 242, 250, 278 nm; CD ($CHCl_3$) $[\theta]_{295.4}$ +10.5 × 10^3, $[\theta]_{263.9}$ −12.9 × 10^3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.87 (3H, s), 3.88 (2H, overlapped, H-8), 3.90 (3H, s), 6.00 (1H, t, J = 6.4 Hz, H-7), 6.08 (1H, br s), 6.38 (1H, d, J = 15.5 Hz, H-8′′), 6.50 (1H, d, J = 16.0 Hz, H-8′), 6.86 (1H, d, J = 8.0 Hz, H-5), 6.96 (1H, s, H-2), 7.00 (1H, d, J = 8.0 Hz, H-6), 7.35 (1H, m, H-3′), 7.35 (1H, m, H-4′), 7.35 (1H, m, H-5′), 7.35 (1H, m, H-3′′), 7.35 (1H, m, H-4′′), 7.35 (1H, m, H-5′′), 7.49 (1H, m, H-2′′), 7.49 (1H, m, H-6′′), 7.49 (1H, m, H-2′), 7.49 (1H, m, H-6′), 7.63 (1H, d, J = 15.5 Hz, H-7′′), 7.72 (1H, d, J = 16.0 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 44.7 (t, C-8), 55.9 (q, OCH_3-4), 56.0 (q, OCH_3-3), 74.7 (d, C-7), 109.8 (d, C-2), 111.2 (d, C-5), 117.6 (d, C-8′), 119.1 (d, C-6), 120.3 (d, C-8′′), 127.9 (d, C-2′′), 127.9 (d, C-6′′), 128.2 (d, C-2′), 128.2 (d, C-6′), 128.8 (d, C-3′), 128.8 (d, C-5′), 128.9 (d, C-3′′), 128.9 (d, C-5′′), 129.8 (s, C-1), 130.3 (d, C-4′), 130.6 (d, C-4′′), 134.2 (s, C-1′), 134.7 (s, C-1′′), 141.6 (d, C-7′′), 145.8 (d, C-7′), 149.2 (s, C-4), 149.2 (s, C-3), 166.0 (s, C-9′′), 166.5 (s, C-9′); HRESIMS *m*/*z* 480.1821 (calcd for $C_{28}H_{27}NO_5Na$, $[M + Na]^+$, 480.1787).

Synthesis of (−)-*R*-Norepinephrine Derivative (7). Diazomethane was added to a solution of l-norepinephrine **5** (100 mg) in MeOH (200 mL), and the mixture was cooled to −15 °C. The mixture was kept 72 h at −15 °C. A second batch of diazomethane was added, and the mixture was kept for a further 72 h at −15 °C. The solvent was evaporated with a strong stream of N_2.

The crude methylated product (**6**) was dried and dissolved in pyridine (2 mL). Cinnamoyl chloride (207 mg, 2.1 equiv) was added, and the reaction mixture was gently heated (40 to 45 °C) for 16 h. The excess pyridine was removed by a strong stream of N_2. The mixture was separated by preparative TLC using $CHCl_3$−MeOH, 99:1, to give the (−)-*R*-norepinephrine derivative (**7**). Its ^{1}H NMR spectrum was identical to that of the natural product (**1**), CD ($CHCl_3$) $[\theta]_{298.6}$ −12.5 × 10^3, $[\theta]_{264.3}$ +23.5 × 10^3

Marmesin (2): white crystals, $[\alpha]_D^{25}$ +9.63° (*c* 0.166, $CHCl_3$); IR, UV, HREIMS, and ^{1}H and ^{13}C NMR data identical to literature value.[16]

Decarine (3): yellow crystals; UV, HREIMS, ^{1}H, ^{13}C NMR data identical to reference values.[17−19]

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