# A Bioactive Spirolactone Iridoid and Triterpenoids from *Himatanthus sucuuba*

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***Himatanthus sucuuba*** **is an Amazonian tree with abundant, yet conflicting ethnobotanical information. Investigation of the polar and non-polar constituents led to the isolation of plumericin, a bioactive spirolactone iridoid, and four known pentacylic triterpenes: lupeol acetate, lupeol cinnamate, lupeol $\beta$-phenyl propionate, and $\alpha$-amyrin cinnamate.**

**Key words** *Himatanthus sucuuba*; phytochemistry; plumericin; lupeol ester

*Himatanthus sucuuba*, Spruce (M. Arg.) Woodson (Apocynaceae) ("Bellaco-Caspi"), a tree from the Amazon rain forest, has received much attention in the literature for the treatment of various ailments, although the ethnobotanical information is somewhat contradictory. For example, in Perú, an infusion from the stem bark has been used as wound-healing agent, for the treatment of tumors, boils and swellings, against arthritis, as a vermifuge laxative,[1] and as hallucinogen.[2] In Brazil, on the other hand, the dried bark is alleged to have anti-ulcer and aphrodisiac activities whereas the latex is used as an antitumor agent.[3] The Caboclos in the Amazon use the dried stem bark for its analgesic and anti-tussive activities.[4] In Colombia, the root is said to be very poisonous.[5] The toxicity of the reproductive system and teratogenic potential of the stem bark have been investigated.[6] Recently, two lichen depsides found in the bark of *H. sucuuba*, have shown inhibition against monoamine oxidase-B.[7] The *in vivo* cicatrizant activity of *H. sucuuba* has been investigated by our group. Although, in an initial experiment, an extract of the bark of *H. sucuuba* showed significant cicatrizant activity, this result could not be reproduced in subsequent runs.[8] An acute toxicity screening—after i.p. administration of 0.1 to 1 mg of extract per gram of mouse weight—determined that *H. sucuuba* was non-toxic in this range.

As part of our systematic search for potential anti-cancer agents from plants,[9] an extract of the bark of *H. sucuuba* underwent a mechanism-based bioassay fractionation. Our assay uses engineered yeasts which lack the RAD52 DNA repair pathway, one of the three major DNA repair pathways that have been defined in yeast, and may also lack the gene for topoisomerase I.[10] An extract which inhibits these yeast mutants to a greater extent than it inhibits the wild-type strain RS188N is thus expected to contain agents which induce DNA damage.

## Results and Discussion

The ethanolic extract of the bark of *H. sucuuba* [$IC_{12}$= 4000 $\mu$g/ml (RS321)] was partitioned between hexane and 80% aq. methanol, resulting in enhanced activity in the latter fraction. Further partitioning (60% aq. methanol/$CH_2Cl_2$) concentrated the activity in the dichloromethane fraction ($IC_{12}$=380 $\mu$g/ml). Chromatographic purification of this fraction led to the isolation of a known iridoid lactone, plumericin (**1**),[11] responsible for the DNA damaging activity of the extract. The activity observed was relatively weak, with an $IC_{12}$ value of 70 $\mu$g/ml for the RS321 yeast strain. For comparison purposes, the known active agent streptonigrin[12] has an $IC_{12}$ value of 0.65 $\mu$g/ml against RS321. Compound **1** is a known antifungal which has been isolated previously from *H. sucuuba*[7] and other *Himatanthus* species.[13] The non-polar (hexane) fraction was investigated for its *in vitro* differential cytotoxic activity using various cancer cell lines (H460, ME180, DU145, MCF-7, HT29). The resulting 50% growth inhibition ($GI_{50}$) values were greater than the control (BALB/c 3T3, $GI_{50}$ 0.125 mg/ml) for all cell lines except for minor activity against human non-small lung cell carcinoma (H-460, $GI_{50}$ 0.080 mg/ml). Chromatographic purification led to the isolation of four pentacyclic triterpenes. Preliminary experimental NMR data signaled the presence of one ursane and three lupane triterpenoids. Further analysis, and comparison with literature data,[14] confirmed the presence of $\alpha$-amyrin cinnamate (**2**),[15] and the acetate (**3**),[16] cinnamate (**4**),[17] and $\beta$-phenyl propionate (**5**)[18] esters of lupeol. Because the $^{13}$C-NMR data for **2**, **4** and **5** have not been re-

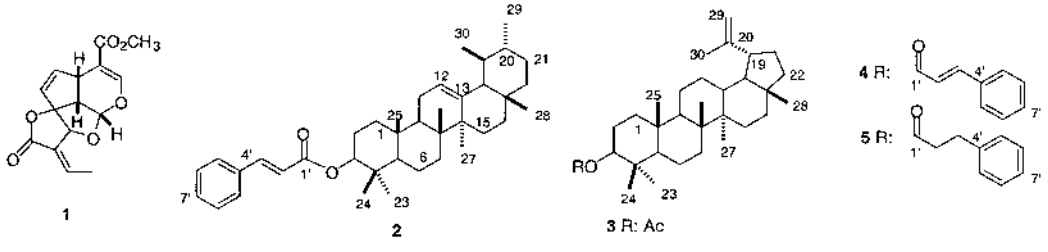


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Table 1. Assignment of $^{13}$C-NMR Chemical Shift (ppm) for **2**, **4**, **5**

| C No. | 2 | 4 | 5 |
|---|---|---|---|
| 1 | 38.49 | 38.43 | 38.37 |
| 2 | 23.70 | 23.82 | 23.68 |
| 3 | 81.02 | 81.06 | 81.06 |
| 4 | 37.96 | 37.84 | 37.82 |
| 5 | 55.31 | 55.44 | 55.38 |
| 6 | 18.26 | 18.23 | 18.19 |
| 7 | 23.88 | 34.23 | 34.21 |
| 8 | 40.05 | 40.87 | 40.86 |
| 9 | 47.65 | 50.37 | 50.34 |
| 10 | 36.83 | 37.13 | 37.08 |
| 11 | 17.50 | 20.97 | 20.95 |
| 12 | 124.33 | 25.12 | 25.11 |
| 13 | 139.63 | 38.06 | 38.05 |
| 14 | 42.08 | 42.85 | 42.83 |
| 15 | 28.75 | 27.45 | 27.44 |
| 16 | 28.61 | 35.58 | 35.57 |
| 17 | 33.75 | 43.00 | 43.00 |
| 18 | 59.07 | 48.31 | 48.29 |
| 19 | 39.65[a)] | 48.02 | 48.01 |
| 20 | 39.61[a)] | 150.98 | 150.98 |
| 21 | 31.25 | 29.85 | 29.84 |
| 22 | 41.54 | 40.01 | 40.00 |
| 23 | 28.10[b)] | 28.02 | 27.89 |
| 24 | 16.87[c)] | 16.67 | 16.53 |
| 25 | 15.75 | 16.21 | 16.16 |
| 26 | 16.90[c)] | 16.00 | 15.97 |
| 27 | 23.25[d)] | 14.54 | 14.52 |
| 28 | 28.13[b)] | 18.01 | 18.01 |
| 29 | 23.39[d)] | 109.35 | 109.35 |
| 30 | 21.39 | 19.30 | 19.29 |
| 1′ | 166.80 | 166.83 | 172.73 |
| 2′ | 144.26 | 118.90 | 29.70 |
| 3′ | 118.87 | 144.23 | 31.10 |
| 4′ | 134.56 | 134.58 | 126.18 |
| 5′ | 128.03 | 128.03 | 128.26 |
| 6′ | 128.83 | 128.84 | 128.44 |
| 7′ | 130.11 | 130.11 | [e)] |

*a*—*d*) Four sets of interchangeable signals. *e*) Not observable.

ported in the literature, we have appended them in Table 1. Compound **3** has been associated with various biological activities,[19] including significant inhibition of tumor growth induced by both, tumor initiators and tumor promoters.[20] We tested **3** against the same battery of tumor derived cell lines (*vide supra*), but found no observable cytotoxic activity ($GI_{50}$ >1 mg/ml for all cell lines).

**Experimental**

Melting points are uncorrected. The $^1$H- and $^{13}$C-NMR spectra were recorded on a Varian Unity 400 or a Bruker AC 300 MHz spectrometer using deuteriochloroform as solvent with 0.03% tetramethylsilane (TMS) as an internal standard. Mass spectra were determined with a VG Quattro instrument. Column chromatography was performed using silica gel (Aldrich, 70—230 mesh, 60A). Preparative TLC was performed using E. Merck Silica Gel 60 F254. Analytical TLC was performed using Macherey-Nagel Polygram Sil G/UV254 precoated plates. High-resolution mass spectra (HR-MS) were recorded at the Midwest Center for Mass Spectrometry of the University of Nebraska-Lincoln.

**Isolation** Samples of *Himatanthus sucuuba* were collected in the vicinity of the Amazonas and Nanay Rivers in the Department of Iquitos, Peru. A complete voucher specimen, FA6251, identified by Franklin Ayala, was deposited in the Herbarium Amazonensis in Iquitos, Peru. Maceration of the bark (1 kg) with EtOH gave 8.8 g of extract. An aliquot (1.9 g) was partitioned between hexane and 80% aq. MeOH, and the methanol soluble fraction was further partitioned between $CH_2Cl_2$ and 60% aq. MeOH. Purification of the $CH_2Cl_2$ fraction (147 mg) using chromatography (8.1 g $SiO_2$, 3% MeOH in $CH_2Cl_2$) led to the isolation of **1** (2.2 mg). This compound was identified by comparing its $^{13}$C- and $^1$H-NMR spectra with published data.[11] Another aliquot (1.24 g) of the crude ethanol extract was subjected to column chromatography ($SiO_2$) and a non-polar eluent system (hexane/toluene 3 : 1) to give **3** (205 mg), and three slightly more polar spots, which were separated using preparative TLC ($SiO_2$, hexane/toluene 1 : 4), to give **2** (43 mg), and a partially separated mixture of **4** (16 mg), and **5** (8 mg). Bioassays were conducted using published procedures.[9]

$\alpha$-Amyrin Cinnamate (**2**): Crystals, mp 97—100 °C, HR-MS: 556.4271 ($M^+$, deviation −1.76 ppm).

Lupeol Acetate (**3**): Crystals, mp 206—209 °C (lit.[21] 214—215 °C), HR-MS: 468.3971 ($M^+$, deviation +0.74 ppm).

Lupeol Cinnamate (**4**): Amorphous solid.

Lupeol $\beta$-Phenylpropionate (**5**): Amorphous solid.

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