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Sipaucins A–C, sesquiterpenoids from *Siparuna pauciflora*☆

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Abstract

The phytochemical investigation of the leaves of *Siparuna pauciflora* yielded three novel sesquiterpenoids: the germacrane sipaucin A, the elemane sipaucin B and sipaucin C, comprising a new type of carbon skeleton. In addition, four known aporphine alkaloids—*nor*-boldine, boldine, laurotetanine, and *N*-methyl-laurotetanine—were obtained. The evaluation of the antiplasmodial activity of the isolated compounds against two strains of *Plasmodium falciparum* (PoW, Dd2) showed a moderate activity of *nor*-boldine.

Keywords: *Siparuna pauciflora*; Monimiaceae; Sesquiterpenoids; Aporphine alkaloids; *Plasmodium falciparum*

1. Introduction

Siparuna pauciflora (Beurl.) A.DC. (Monimiaceae, syn.: *Citrosma pauciflora* Beurl.) is a lemon-scented shrub or small tree, distributed in the New World tropics from Costa Rica to Peru and Colombia. Due to its smell, the plant is called "Limoncillo" in Costa Rica (Duke, 1962) and a decoction is used by certain Indians in Panama against fever (Morton, 1981). Previous investigations led to the isolation of the aporphine-type alkaloids nantenine, *N*-methyl-laurotetanine and noroliveroline (Lopez et al., 1988), secondary metabolites quite characteristic for the Monimiaceae (Leitão et al., 1999). Sesquiterpenoids on the other hand have not been well studied and up to now only few derivatives have been reported from three *Siparuna* species (Antonio et al., 1984; El-Seedi et al., 1994; Jenett-Siems et al., 2000).

During our ongoing phytochemical research on antiplasmodial plant species from Central America, we investigated the leaves of *S. pauciflora* and isolated three novel sesquiterpenes together with four known aporphine alkaloids.

2. Results and discussion

Analysis of a crude alkaloid fraction from the leaves of *S. pauciflora* yielded the aporphines *nor*-boldine, boldine, laurotetanine, and *N*-methyl-laurotetanine which were identified by comparison of their spectroscopic data (^{1}H NMR, EIMS) with literature values (Guinaudeau et al., 1975; Shamma and Rothenberg, 1978) as well as by NOE experiments. These compounds are quite common within the Monimiaceae (Leitão et al., 1999), but with the exception of *N*-methyl-laurotetanine have not been reported for *S. pauciflora* before.

A lipophilic extract (petrol ether/EtOAc 1:1) of the dried leaves upon separation by column chromatography and preparative HPLC yielded three compounds, the structures of which were elucidated by spectroscopic means. The EIMS spectrum of **1** displayed a molecular ion peak at m/z 366, a characteristic

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fragment at m/z 307 pointed to the loss of an acetate moiety. The molecular formula was determined as $C_{19}H_{26}O_7$ (HR-EIMS). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed the occurrence of two acetate moieties and two additional methyl groups: δ 1.28 and 1.89. The latter, obviously olefinic, displayed HMBC correlations (Table 2) to a quaternary olefinic carbon atom at δ 136.7 as well as to a carbonyl signal at δ 204.0. The other methyl group showed long-range correlations to two quaternary oxygenated carbons (δ 60.5; 62.9) and to an oxygenated methylene group (δ 64.7). The chemical shift of the methylene protons in the ^1H NMR (δ 4.16; 4.12) hinted at an esterification, probably with one of the acetyl moieties. In addition, in the HMBC spectrum long-range correlations of an isolated methylene group (δ 3.53, *d*, $J = 14.0$ Hz; δ 2.89, *d*, $J = 14.0$ Hz) to the carbon atoms at δ 60.5 and 62.9 as well as to the carbonyl group at δ 204.0 were observed. Decoupling experiments revealed that the olefinic proton at δ 6.07 was coupled to a methylene group, which was again coupled to another methylene group. Finally, an oxygenated methylene group, showing HMBC correlations to one of the acetyl moieties, one quaternary and one tertiary oxygenated carbon atom and one additional methylene group coupled to a hydroxymethine proton were observed. From these data, **1** (sipaucin A) was identified as a highly oxygenated germacrane derivative. The relative stereochemistry was deduced from NOE experiments. Irradiation in the olefinic proton signal caused an enhancement of H-5 and H-8a, whereas irradiation in H-8b led to an enhancement of the signal of H-12. The olefinic methyl group as well as the C-11 methyl group on the other hand showed NOE correlations to H-15 (Fig. 1).

Table 1
^1H NMR data (400 MHz, $CDCl_3$) of sipaucins A–C (**1**–**3**)

H	**1**	**2**	**3**
1	6.07 *dq* (12.0; 2.0)	5.83 *dd* (11.0; 17.0)	3.36 *br s*
2	2.44 *m*	5.02 *d* (17.0)	1.64 *m*
	2.60 *m*	5.15 *d* (11.0)	1.97 *m*
3	2.60 *m*	5.02 *br s*	1.20 *m*
	1.27 *m*	5.19 *br s*	1.32 *m*
5	3.29 *dd* (10.0; 6.0)	2.73 *t* (5.5)	3.01 *dd* (2.5; 7.0)
6	2.74 *dd* (15.0; 6.0)	2.44 *dd* (5.5; 18.0)	2.88 *ddd* (19.0; 7.0; 2.0)
	1.40 *dd* (15.0; 6.0)	2.59 *ddd* (5.5; 18.0; 1.5)	2.47 *dt* (19.0; 2.0)
8	3.53 *d* (14.0)	6.30 *br s*	6.14 *t* (2.0)
	2.89 *d* (14.0)		
12	4.16 *d* (12.0)	4.05 *d* (12.0)	4.25 *d* (11.5)
	4.12 *d* (12.0)	4.27 *d* (12.0)	4.18 *d* (11.5)
13	1.28 *s*	1.37 *s*	1.46 *s*
14	1.89 *d* (2.0)	1.17 *s*	1.49 *s*
15	4.56 *d* (13.0)	4.41 *d* (14.0)	4.68 *d* (10.5)
	3.69 *dd* (13.0; 1.0)	4.45 *d* (14.0)	4.15 *d* (10.5)
2′	2.10 *s*[a]	2.05 *s*[a]	2.08 *s*[a]
2″	2.13 *s*[a]	2.08 *s*[a]	2.09 *s*[a]

[a] Interchangeable.

Table 2
^{13}C NMR data (100 MHz, $CDCl_3$) and HMBC correlations of sipaucins A–C (**1**–**3**)

C	**1**		**2**		**3**	
	δ_C (ppm)	HMBC correlations	δ_C (ppm)	HMBC correlations	δ_C[a] (ppm)	HMBC correlations
1	138.2		140.9	2, 5, 14	67.0	2, 3, 14
2	34.3		115.2		28.2	1, 3
3	31.9		115.8	5, 15	29.0	1, 2, 5, 15
4	61.9	6, 15	143.2	5, 6, 15	48.5	2, 3, 5, 14, 15
5	61.5		47.0	1, 3, 6, 14, 15	47.1	3, 6, 8, 15
6	24.0		29.1	5, 8	32.0	5, 8
7	60.5	8, 12, 13	162.5	5, 6, 12, 13	181.3	6, 8, 12, 13
8	42.6		124.1	6	130.8	6
9	204.0[a]	8, 14	201.1	5, 14	208.2	5, 8
10	136.7	8, 14	50.8	1, 2, 5, 6, 8, 14	68.0	2, 3, 14, 15
11	62.9	8, 12, 13	74.0	8, 13	73.6	8, 12, 13
12	64.7	13	69.4	13	70.1	13
13	15.6	12	23.6	12	25.0	
14	12.6	9, 10	17.8		16.5	
15	62.9		67.2	3, 5	66.7	
1′	170.2	2′, 12	170.6	2′, 12	172.1	2′, 12
2′	20.6		20.9		21.2	
1″	170.3	2″, 15	170.8	2″, 15	172.1	2″, 15
2″	20.6		20.7		21.2	

[a] Data from HMQC and HMBC spectra.

Compound **2** possessed a molecular ion peak at m/z 350 in the EIMS. The fragment at m/z 290 was again due to the loss of acetic acid. The molecular formula of $C_{19}H_{26}O_6$ (HR-EIMS) suggested a sesquiterpenoid containing two acetyl groups. The ^1H NMR spectrum displayed characteristic signals for a vinyl group: δ 5.02 (1H, *d*, $J = 17.0$ Hz), 5.15 (1H, *d*, $J = 11.0$ Hz) and 5.83 (1 H, *dd*, $J = 11.0$ Hz, 17.0 Hz). In the HMBC, a methyl group at δ 1.17 (3H, *s*) showed correlations to the vinyl group, to a carbonyl group (δ 201.1), to a quaternary carbon (δ 50.8) as well as to a methine group at δ 47.0. The ^1H signal at δ 2.73 corresponding to the latter showed correlations to an exomethylene group (δ 115.8 and 143.2) and to a hydroxymethylene group (δ 67.2), which also showed long-range couplings to the

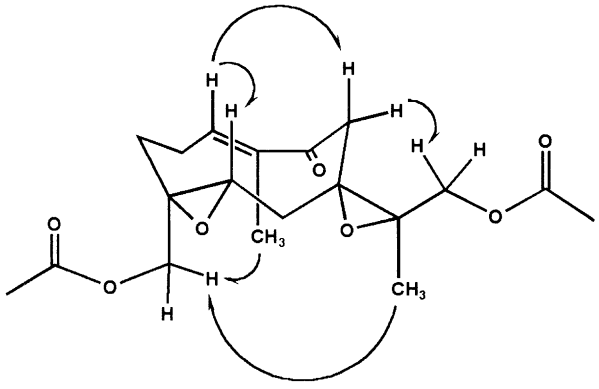


Fig. 1. Selected NOE correlations of sipaucin A (**1**).

exomethylene protons. Furthermore it was coupled to a methylene group at δ 2.44 and 2.59 which showed an allylic coupling to an olefinic proton at δ 6.25. This proton displayed HMBC correlations to the quaternary carbon at δ 50.8 thus suggesting a ring closure. The side-chain at the tetra-substituted olefinic carbon (δ 162.5) was composed of a quaternary oxygen-bearing carbon atom (δ 74.0), one methyl group (δ 23.6) and one hydroxymethylene group (δ 69.4). From these data, **2** (sipaucin B) was identified as an elemane derivative. The positions of the acetyl moieties were deduced from the HMBC spectrum. The relative stereochemistry at C-5 and C-10 was assigned by NOE experiments. The coupling constant $J = 5.5$ Hz for H-5 required an equatorial position. The methyl group at C-10 possessed NOE correlations to H-5 and H-6 axial. Thus, this methyl group must be in an axial position, leading to a *cis*-elemane (Fig. 2). The stereochemistry at C-11 cannot be resolved by spectroscopic means.

The EIMS of compound **3** displayed a molecular ion peak at m/z 366, corresponding to a molecular formula of $C_{19}H_{26}O_7$ (HR-EIMS). ^1H and ^{13}C NMR spectra showed again two acetyl moieties as well as two methyl groups and two hydroxymethylene groups. The methyl group at δ 1.49 possessed long-range correlations to an oxygen-bearing quaternary carbon atom (δ 68.0), a quaternary carbon (δ 47.1) which was attached to a hydroxymethylene group, and a methine group (δ 67.0). The corresponding proton at δ 3.36 showed HMBC cross-peaks to two methylene groups which were correlated to the quaternary carbon at δ 68.0. These data allowed the identification of one part of **3** as a substituted five-membered ring. One of the methylene groups displayed an additional long-range correlation to a methine group at δ 48.5. The corresponding proton was located at δ 3.01 (*dd*, $J = 2.5$ Hz; 7.0 Hz). It showed HMBC correlations to a carbonyl moiety (δ 208.2) and a methylene group (δ 32.0). Further interpretation of the HMBC spectrum led to a cyclopentenone sub-structure with an identical side-chain as **2**. The positions of the acetyl moieties were again deduced from the HMBC spectrum. Combination of both fragments led to **3** (sipaucin C) with an unusual re-arranged sesquiterpenoid skeleton. As already mentioned, the stereochemistry of **1** and **2** was deduced from the results of the NOE spectroscopy. In the case of compound **3** the indicated stereochemistry was supported by the possible biogenetic formation. All these compounds are biogenetically derived from germacra-1(10),4,7(11)-triene. Oxidation at C-9 and C-15 and epoxidation of the 7(11)-doublebond leads to a germacra-1(10),4-diene derivative which acts as precursor for **1**–**3**. Epoxidation of the 4,5-doublebond gives compound **1** while the Cope rearrangement of the 7,9-enone leads to compound **2**. 1(10)-Epoxidation of the 7,9-enone and subsequent cyclization and rearrangement of the intermediate eudesmane derivative would finally give compound **3**. The indicated stereochemistry is deduced from this biogenetic pathway but was not proved further.

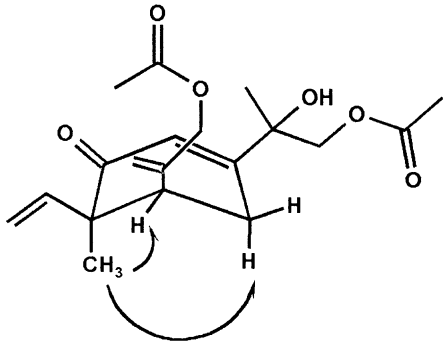
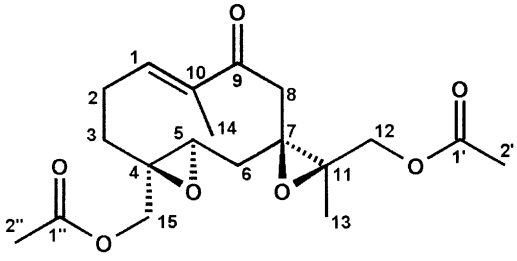
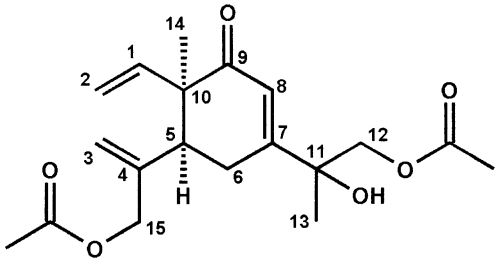


Fig. 2. Selected NOE correlations of sipaucin B (**2**).



1



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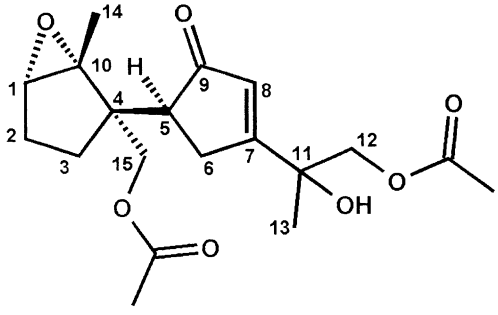

3

Compounds **1**–**3** are the first sesquiterpenoids obtained from this species. Up to now, only simple mono- and sesquiterpenoids such as β-elemene or calamenene derivatives were described for the Monimiaceae, obviously constituents of the widespread essential oils (Leitão et al., 1999). Substances **1**–**3** on the other hand, are highly oxygenated members of this class of compounds with sipaucin C (**3**) being the most unusual one and possessing a novel type of carbon skeleton.

Evaluation of the antiplasmodial activity of the isolated compounds against two strains of *Plasmodium falciparum* (PoW, Dd2) showed that only *nor*-boldine possessed a moderate activity [IC_{50} values: 3.1 μg/ml (Pow), 5.4 μg/ml (Dd2)], whereas the other aporphines as well as the sesquiterpenoids proved to be inactive.

3. Experimental

3.1. General

For fractionation silica gel 60 (70–230 mesh) was utilized. Preparative high-performance liquid chromatography (HPLC) was performed on a Knauer instrument equipped with Eurochrom 2000 on a Nucleosil P 300 C-18 (10 μm) reversed-phase column. EIMS were recorded on a Finnigan MAT CH7A (70 eV), HR-EIMS on a Finnigan MAT 711 (80 eV). ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOE spectra were obtained on a Bruker AVANCE DPX 400 MHz or a Bruker DRX 500 MHz spectrometer (TMS as int. standard; $CDCl_3$ as solvent).

3.2. Plant material

Leaves of *S. pauciflora* were collected near Cerro Caracoral in Central Panama on 3 March 1997 and identified by Professor M.D. Correa A., Director of the Herbarium of the Universidad de Panama, where voucher specimens (FLORPAN 2753, PMA) are deposited.

3.3. Extraction and isolation

Ground dried leaves of *S. pauciflora* (200 g) were extracted four times with 1 l petrol ether/EtOAc 1:1, each. After evaporation of the solvent the residue (6 g) was fractionated by column chromatography on silica gel with cyclohexane, cyclohexane/EtOAc mixtures of increasing polarity (9:1, 8:2, 7:3, 1:1, 3:7) and EtOAc. Fraction 9 which was eluted with cyclohexane/EtOAc 1:1 was further purified by prep. HPLC (H_2O/MeOH 70:30→30:70 after 50 min) and yielded **1** (R_t = 35 min, 4 mg) and **2** (R_t = 40 min, 10 mg). Fraction 11 which was eluted with cyclohexane/EtOAc 3:7 was again purified by prep. HPLC (H_2O/MeOH 7:3) and gave 4 mg of **3** (R_t = 35 min).

The extracted plant material was then air-dried and again extracted three times with 1 l MeOH (80%), each. After evaporation of the solvent, the residue was redissolved in an aq. solution of tartaric acid (2%) and extracted three times with 400 ml CH_2Cl_2, each. Afterwards the aq. phase was alkalinized with aq. NH_3 (10%) and again extracted four times with 400 ml CH_2Cl_2, each. The crude alkaloids were fractionated by prep. HPLC (0.5% H_3PO_4/MeOH 85:15→50:50 after 50 min). Peak I (R_t = 15 min) and peak II (R_t = 25 min) were further purified by prep. DC (acetone/toluol/EtOH/NH_3 45:45:7:3). Separation of peak I yielded 20 mg of *nor*-boldine (R_f: 0.17) and 10 mg of boldine (R_f: 0.33) whereas from peak II 25 mg of laurotetanine (R_f: 0.19) and 12 mg of *N*-methyl-laurotetanine (R_f: 0.35) were obtained.

3.3.1. Sipaucin A (*1*)

Yellow oil. $[\alpha]_D^{20}$ −4 ($CHCl_3$, *c* 0.10); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m/z* (rel. int.) 366 $[M]^+$ (1), 307 (10), 293 (15), 109 (69); HR-EIMS: *m/z* 366.1675 (calc. for $C_{19}H_{26}O_7$ 366.1678), 293.1388 (calc. for $C_{16}H_{21}O_5$ 293.1389).

3.3.2. Sipaucin B (*2*)

Yellow oil. $[\alpha]_D^{20}$ +8 ($CHCl_3$, *c* 0.25); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m/z* (rel. int.) 350 $[M]^+$ (11), 332 (7), 290 (16), 277 (21), 272 (15), 233 (44), 184 (68), 142 (100), 124 (63), 106 (76), 91 (77); HR-EIMS: *m/z* 350.1728 (calc. for $C_{19}H_{26}O_6$ 350.1729), 332.1622 (calc. for $C_{19}H_{24}O_5$ 332.1624), 290.1514 (calc. for $C_{17}H_{22}O_4$ 290.1518).

3.3.3. Sipaucin C (*3*)

Yellow oil. $[\alpha]_D^{20}$ +3 ($CHCl_3$, *c* 0.30); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m/z* (rel. int.) 366 $[M]^+$ (3), 293 (25), 180 (50), 125 (100), 109 (81); HREIMS: *m/z* 366.1677 (calc. for $C_{19}H_{26}O_7$ 366.1678), 293.1377 (calc. for $C_{16}H_{21}O_5$ 293.1389).

3.4. In vitro antiplasmodial activity

The bioassay was performed as described earlier (Kraft et al., 2000). The chloroquine-sensitive strain PoW (IC_{50} for chloroquine×2 H_3PO_4 = 0.015 μM) and the chloroquine-resistant clone Dd2 (IC_{50} for chloroquine×2 H_3PO_4 = 0.14 μM) of *P. falciparum* were maintained in continuous culture as described by Trager and Jensen (1976). The antiplasmodial assay was carried out according to the method of Desjardins et al. (1979) and activities were determined by a ^{3}H-hypoxanthine-incorporation assay.

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