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Bolivianine, a New Sesterpene with an Unusual Skeleton from *Hedyosmum angustifolium,* and Its Isomer, Isobolivianine

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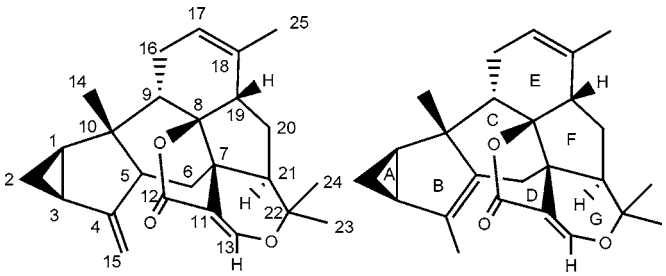
ABSTRACT



Bolivianine, a novel sesterpene with an unprecedented skeleton, has been isolated from the trunk bark of *Hedyosmum angustifolium* (Chloranthaceae), with isobolivianine, an isomer formed under acidic conditions. The structure and relative stereochemistry were elucidated on the basis of spectroscopic data. A hypothesis for biogenesis was made.

Hedyosmum angustifolium (Chloranthaceae) is a small tree growing in the humid high montane tropical Andean forest of Bolivia, Peru, and Ecuador. The rarity of chemical data about the genus *Hedyosmum* motivated us to study the composition of *H. angustifolium* in our ongoing program of research for new bioactive compounds in the Bolivian flora. The program started with the organization of six permanent sampling plots in different mountain forests between 1600 and 2700 m on the east side of the Andean Cordillera in Bolivia, in The Cotapata National Park near to La Paz.

The only previous chemical study on *H. angustifolium* concerned its essential oil composition,[1] and there are only a few phytochemical studies on the genus *Hedyosmum*. Flavonoid glycosides[2] were isolated, as well as sesquiterpene lactones: 7,10-epoxyhedyosminolide, a guainolide, was isolated from *H. arborescens*,[3] and the onoseriolide, a lindenane, was isolated from *H. brasiliense*.[4] Lindenane sesquiterpene lactones are widely distributed in the Chloranthaceae family, as shown by numerous publications by Kawabata et al.,[5] who also described some new skeletons in this family such as

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(1) Lorenzo, D.; Loayza, I.; Dellacassa, E. *Flavour Fragrance J.* **2003**, *18*, 32−35.
(2) Cardenas, L. C.; Rodriguez, J.; Villaverde, M. C.; Riguera, R.; Cadena, R.; Otero, J. A. *Planta Med.* **1993**, *59*, 26−27.
(3) Bercion, S.; Couppe, de K/Martin, M.-A.; Baltaze, J.-P.; Bourgeois, P. *Fitoterapia* **2005**, *76*, 620−624.
(4) Trentin, A. P.; Santos, A. R. S.; Guedes, A.; Pizzolatti, M. G.; Yunes, R. A.; Calixto, J. B. *Planta Med.* **1999**, *65*, 517−521.

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Table 1. NMR Data for Compound **1** (C_6D_6)[a]

	δC	δH	mult. (*J* in Hz)		HMBC (H→C)	COSY	NOESY
1	***26.1***[b]	1.46	m	CH		2a, 2b, 3, or 6b	2a, 16b, 9
2a	16.3	0.70	ddd: 5.1; 8.5; 9.0	CH2	3 or 16 (w), 4 (w), 10 (w)[c]	1, 2b, 3, or 6b	1, 2b, 3, or 6b
2b		0.91	m			1, 2a, 3, or 6b	2a, 14
3	**23.5**	**1.94**	**m**	CH	**7, 8, 10, 21 (w)**	**1**, **2a**, **2b**, **6a**	**2a**, **5**, **6a**, **15a**, **21**, **23**
4	152.8			C			
5	52.1	2.55	bdd: 2.1; 12.7	CH	1 or 6 (w), 4, 10, 14, 15 (w)	6a, 15a, 15b	3 or 6b, 16a, 21
6a	***26.2***	1.35	dd: 12.9; 14.2	CH2	5, 11, 21	5, 6b	3 or 6b, 14
6b		**1.94**	**dd: 2.2; 14.2**		**7, 8, 10, 21 (w)**	**1, 2a, 2b, 6a**	**2a, 5, 6a, 15a, 21, 23**
7	52.5			C			
8	96.9			C			
9	46.5	2.30	m	CH	5, 8, 10, 14 (w)	16a	14, 1, 16a
10	39.8			C			
11	110.3			C			
12	168.5			C			
13	149.4	7.39	s	CH	7, 11, 12, 22		
14	20.5	1.00	s	CH3	1 or 6, 2, 5, 9, 10		2b, 6a, 9
15a	105.4	4.74	bs	CH2	3 or 16, 5	5, 15b (w)	3 or 6b, 15b
15b		5.18	bs		3 or 16, 5	5, 15a (w)	15a
16a	**23.4**	1.73	m	CH2		9, 16b	5, 16b
16b		2.28	m		8, 9 (w), 17, 18	16a, 17	1, 16a, 17
17	120.5	5.63	m	CH	3 or 16, 9 (w), 19	16b, 25	16b, 25
18	140.0			C			
19	51.6	2.63	dd: 8.1; 12.0	CH	8, 9, 17, 18, 20, 25	20a, 20b	20b, 25
20a	35.3	1.10	m	CH2		19, 20b, 21	20b, 21
20b		2.07	ddd: 6.8; 12.0; 14.0		8, 19, 21, 22	19, 20a, 21	19, 20a, 24
21	44.3	1.69	dd: 6.9; 11.2	CH	7, 11, 20	20a, 20b	5, 20a
22	78.4			C			
23	27.2	1.16	s	CH3	21, 22, 24	24	3 or 6b, 21, 24
24	27.3	1.01	s	CH3	21, 22, 23	23	20b, 21, 23
25	22.5	1.62	bt: 1.9	CH3	17, 18, 19	17	17, 19

[a] Spectra recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C. [b] Ambiguous signals are highlighted in bold or italic. [c] The (w) indicates a weak signal.

the shizukaols (sesquiterpene dimers)[6] or sesquiterpene trimers.[5]

In this paper, we describe the isolation and structure elucidation of bolivianine (**1**) and isobolivianine (**2**), new sesterpenes with an unprecedented skeleton. Biosynthesis from onoseriolide (**3**), a sesquiterpene lactone already isolated from the genus *Hedyosmum* and other Chloranthaceae is proposed.

Two samples of the plant material consisting of trunk bark of *H. angustifolium* were collected in August 2004 and August 2005 on two different trees in the plots of the Cotapata National Park, Bolivia. Each sample (1 kg) was air-dried and powdered.

The ethyl acetate extract of the first sample was submitted to successive flash column chromatography on silica gel; 5.4 mg of a pale yellow oil was obtained. A first ^{1}H NMR was run in deuterated chloroform, then the solvent was removed and the sample stored at room temperature, before a second ^{1}H NMR spectra and more detailed NMR could be run. Isobolivianine (**2**) of 80% purity was identified. Additional purification of **2** gave pure isobolivianine. Still, we noticed some changes between the first and the second ^{1}H spectra. To confirm the isolation of this unusual sesterpene, the second sample of plant material was submitted to the same purification scheme. Six milligrams of a product with the same TLC profile and mass spectra as isobolivianine was isolated. To obtain complementary NMR data, and especially to avoid any signal overlap hindering the determination of the relative stereochemistry of isobolivianine, NMR was run in deuterated benzene, which unexpectedly enabled the structure of bolivianine (**1**) to be determined. Indeed, **1** was also isolated the first time, as shown by the first ^{1}H NMR, but it was isomerized when we recorded the second ^{1}H spectra. We reproduced this isomerization by dissolving bolivianine **1** in deuterated chloroform and removing the solvent under reduced pressure. This was an expected double bond migration in acidic medium, leading to the most substituted alkene. So **1** was the natural compound and **2** an artifact. Kawabata et al. already described such an acid-catalyzed isomerization of shizukanolide,[7] a lindenane sesquiterperne lactone isolated from *Chloranthus japonicus* (Chloranthaceae), structurally related to bolivianine.

The structure of **1** ($C_{25}H_{30}O_3$, HRTOFESIMS)[8] was determined by careful analysis of its NMR data, which are

(5) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1997**, *47*, 231−235 and refs cited therein.

(6) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121−125.

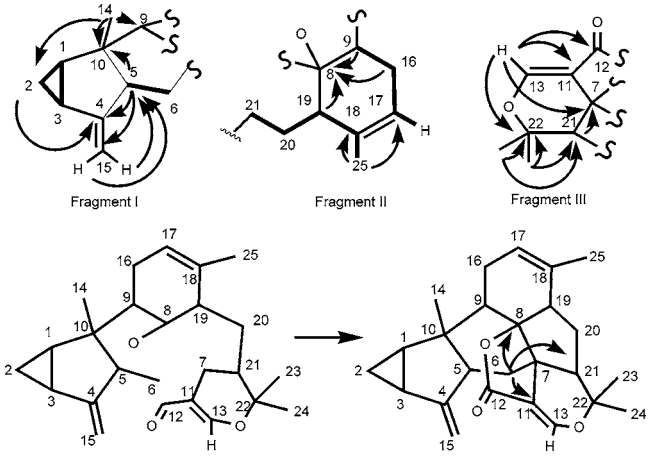
(7) Kawabata, J.; Tahara, S.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *45*, 1447−1453.

(8) Compound **1**: $[\alpha]^{25}_D$ −50 (*c* 0.2, $CHCl_3$); IR (KBr) 2929, 2849, 1752, 1643, 1449, 1384, 1215; HRTOFESIMS *m/z* 379.2285 ($M + H^+$, calcd for $C_{25}H_{31}O_3$ 379.2273).

summarized in Table 1. **1** displayed 25 carbons in the ^{13}C NMR spectrum: one carbonyl from a carboxylic acid derivative, three sp^2 quaternary carbons, one sp^2 methylene, two sp^2 methines, four sp^3 quaternary carbons, six sp^3 methines, four sp^3 methylenes, and four methyls. The ^1H NMR spectrum indicated an ethylenic proton at 5.63 ppm, two ethylenic geminal protons at 4.74 and 5.18 ppm, and a singlet at 7.39 ppm.

The construction of molecule **1** is summarized in Scheme 1. The structure of three fragments was identified thanks to

Scheme 1. Construction of Molecule **1**[a]



[a] Thick bond: COSY correlation; arrow: HMBC correlation.

COSY and HMBC correlations and connected to one another through C9 and C21. To close the structure, we used the HMBC correlations of H6a and H6b, which were no longer ambiguous with H1 because of the distance between the two positions. The lactone between C8 and C12 closed the structure.

The structure of **2** ($C_{25}H_{30}O_3$, HRTOFESIMS)[9] was determined in a similar way. ^1H and ^{13}C NMR spectra of **2** are described in Table 2.

The relative stereochemistry of **1** and **2** was determined by analysis of coupling constants and NOESY experiments, as shown in Figure 1.

For **1**, we assigned the position of the protons and methyl 14 on rings A and B: H1 and H3 were *syn* on ring A, and H1 had NOESY correlation with H2a, so these three protons were *syn* on ring A. Methyl 14 had NOESY correlation with H2b, so it was *anti* to H1and H3 on ring B. Then, the axial or equatorial positions on the ring C were assigned as follows. The coupling constants of H5 and the two H6 showed that H5 was axial, H6a was axial and H6b was equatorial. Methyl 14 had NOESY correlation with H6a, so it was axial, *anti* to H5. Methyl 14 also had NOESY correlation with H9, so H9 was equatorial. Therefore the C9−C16 bond was axial, as confirmed by a NOESY

(9) Compound **2**: $[\alpha]^{25}_D$ −80 (*c* 0.2, $CHCl_3$); IR (KBr) 2927, 2853, 1755, 1646, 1457, 1371, 1223, 1197; HRTOFESIMS m/z 379.2258 ($M + H^+$, calcd for $C_{25}H_{31}O_3$ 379.2273).

Table 2. ^1H and ^{13}C NMR for Compound 2 ($CDCl_3$)[a]

	δC	δH	mult. (*J* in Hz)
1	**27.1**[b]	*1.52*	m
2a	15.1	0.02	m
2b		0.72	ddd: 3.9; 7.4; 8.0
3	26.7	1.65	m
4	136.2		
5	129.8		
6a	**27.2**	**1.97**	m
6b		2.89	d: 14.9
7	54.4		
8	97.8		
9	48.5	**1.97**	m
10	48.6		
11	110.1		
12	170.5		
13	150.0	7.40	s
14	24.5	1.26	s
15	13.7	1.76	d: 1.2
16a	23.5	*1.51*	m
16b		2.37	m
17	121.1	5.57	de: 7.4
18	139.8		
19	50.9	2.51	dd: 6.4; 11.9
20a	36.5	1.37	m
20b		2.26	ddd: 7.9; 12.1; 13.7
21	45.2	2.15	dd: 7.8; 10.1
22	79.4		
23	27.7	1.34	s
24	27.7	1.56	s
25	22.5	1.68	bs

[a] Spectra recorded at 500 MHz for ^1H and 125 MHz for ^{13}C. [b] Ambiguous signals are highlighted in bold and italics.

correlation between H5 and H16a. The C8−C19 had to be equatorial because of the junction between rings C and D. If the C8−C19 bond was equatorial, then the C8−O bond was axial so the C7−C11 bond must be equatorial, *syn* to the methyl 14. This was confirmed by the NOESY correlation between H21 and H5, which also indicated H21 stereochemistry. Furthermore, H21 had NOESY correlation with H20a, H19 had NOESY correlation with H20b, therefore H19 and H21 had to be *anti* on ring F. The use of NOESY effects on five-membered rings often leads to mistakes because these rings are conformationally flexible. In this case, however, the polycyclic structure hindered flexibility. This was confirmed by the coupling constants of H19 and H21 with H20a and H20b, which were higher than usual for a five-membered ring.

For **2**, we saw the same NOESY correlations between H1, H2, H3, and methyl 14, so the stereochemistry around ring A and B was the same as above. The lack of H5 and the overlap between H6a and H9 signals made the assignment of the stereochemistry for ring C more difficult. H16a had NOESY correlation with H21. This was possible only if the C9−C16 and C7−C21 bonds were *anti* to methyl 14 on ring C. Therefore, the stereochemistry around ring C was the same as for **1**. The NOESY effects between H19, H20, and H21 were the same as for **1**, and H21 pointed inside ring C

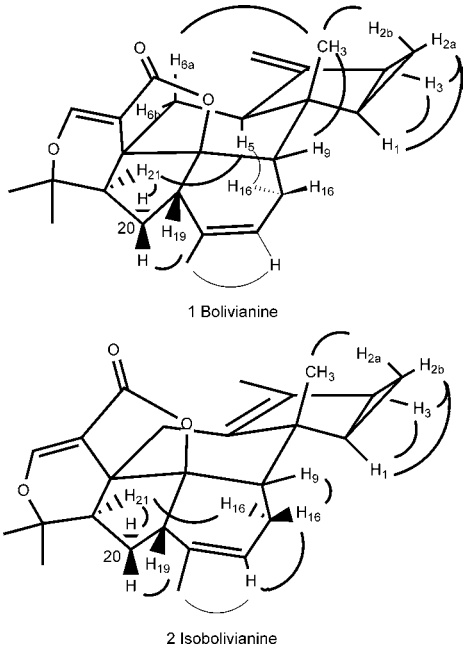


Figure 1. Key NOESY for bolivianine and isobolivianine.

because of its NOESY with H16a. So, as we expected, the relative stereochemistries of **1** and **2** were the same.

Then, we made a hypothesis for the biogenesis of **1**. Retrosynthetic analysis (Figure 2) showed that this sesterpene

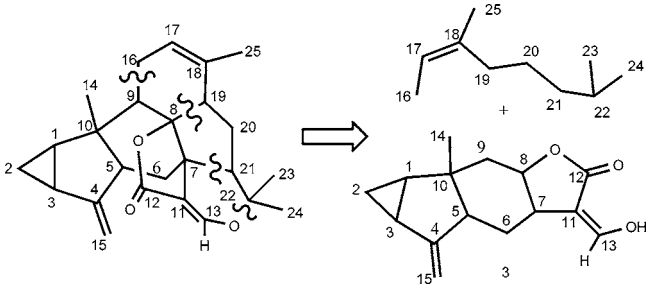
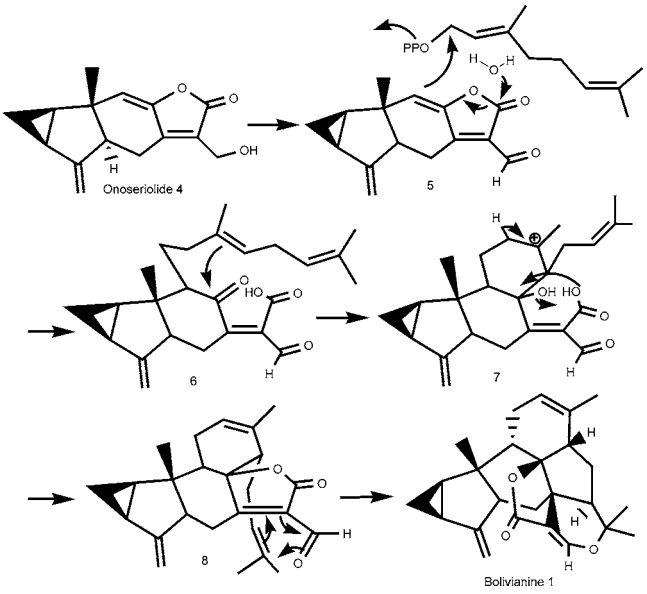


Figure 2. Retrosynthetic analysis of bolivianine.

could be obtained by the condensation of a lindenane sesquiterpene skeleton (**3**) with a geranyl moiety.

In Scheme 2, we propose a possible biogenetic pathway from onoseriolide to bolivianine. After an allylic oxidation, onoseriolide gave aldehyde **5**. Hydrolysis of **5** allowed nucleophilic attack of the enol on a geranylpyrophosphate molecule. After isomerization of the geranyl moiety, **6** was obtained. Nucleophilic attack of the geranyl double bond allowed the formation of the C ring, followed by dehydration giving the lactone **8**. A Diels−Alder reaction gave the G and F ring and compound **1**.

Scheme 2. Hypothesis for the Biogenesis of **1**



Onoseriolide is closely related to shizukanolides, for which the relative and absolute stereochemistries have been determined.[7] The relative stereochemistry we proposed for **1** was in agreement with this previous study, and a hypothesis for the absolute configuration for **1** can be made.

Compounds **1** and **2** displayed no significant activity on *Plasmodium falciparum* or on the MCF7 cell line.

This study is a preliminary report of an unprecedented sesterpene skeleton, found in *Hedyosmum angustifolium*. Other compounds from this plant are currently under investigation by our team and will be reported.

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Supporting Information Available: One- and two-dimensional NMR spectra for bolivianine and isobolivianine. Purification scheme for bolivianine. Experimental procedure for the isomerization of bolivianine. Details for the biological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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