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Eremophilanolides and other constituents from the Argentine liverwort *Frullania brasiliensis*

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Abstract

Two eremophilanolides, 5-epidilatanolides A and B, as well as a new natural bibenzyl were isolated from an Argentine collection of the liverwort *Frullania brasiliensis*, along with the known eudesmane-type sesquiterpene lactones nepalensolide A, nepalensolide B, (+)-frullanolide, and (+)-dihydrofrullanolide, the hopanoid zeorin, the four sterols stigmasta-4,22-dien-3,6-dione, stigmasta-4,22-dien-3-one, stigmasterol, and sitosterol, and a trace amount of atraric acid. The structure and stereochemistry of the eremophilanolides and the bibenzyl were established by a combination of extensive NMR spectroscopy experiments and X-ray crystallographic analysis. Absolute configurations of the new compounds were derived on the basis of CD spectra. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Frullania brasiliensis*; Frullaniaceae; Liverworts; Hepaticae; Eremophilanolides; Eudesmanolides; Hopanoids; Bibenzyls; X-ray analysis; Absolute configuration

1. Introduction

Frullania is a large and complex genus with over 1000 described taxa (Yuzawa, 1991) and the subgeneric and even generic boundaries remain unresolved (von Konrat and Braggins, 2001). *Frullania* species are a rich source of sesquiterpene lactones [causing allergic contact dermatitis (Asakawa et al., 1976; Asakawa, 1982, 1995)], and/or bibenzyl derivatives. By their chemical constitution, they fit into five chemotypes within the Frullaniaceae (Asakawa et al., 1981). The epiphytic stem-leafy species *Frullania brasiliensis* has been placed into chemotype I since previous investigations on the chemistry of collections from Perú (Asakawa and Inoue, 1987) and Ecuador (Nagashima et al., 1991) reported the presence of sesquiterpene lactones as well as other common plant sesquiterpenes. Continuing with the chemical investigation of South American liverworts (Asakawa, 1995; Tori et al., 1996; Bardón et al., 1999a,b), we report the isolation and identification of six sesquiterpene lactones from an Argentine collection of *F. brasiliensis*: the new eremophilanolides **1** and **2**, for which we propose the names 5-epidilatanolides A and B respectively, the previously described nepalensolides **3** and **4** (Tori et al., 1988), and the *ent*-eudesmanolides (+)-frullanolide, **5**, and (+)-dihydrofrullanolide, **6**, (Asakawa, 1982). In addition, the following constituents were isolated in small amounts: the bibenzyl **7**, previously obtained by synthesis (Ronlán et al., 1973), atraric acid, **8**, (Cooke and Down, 1971), the hopanoid zeorin, **9**, and the steroids stigmasta-4,22-dien-3,6-dione (Itokawa et al., 1973), stigmasta-4,22-dien-3-one (Slatkin et al., 1975), stigmasterol, and sitosterol. Absolute configurations of the new compounds were derived on the basis of CD spectra. The method has been frequently employed and some rules derived to rationalize the observed values for the Cotton effect (Waddell et al., 1969; Stöcklin et al., 1970). Particularly, it has shown to be a useful technique to establish the absolute stereostructures for many *cis*-fused γ-lactones isolated from liverworts (Asakawa, 1982).

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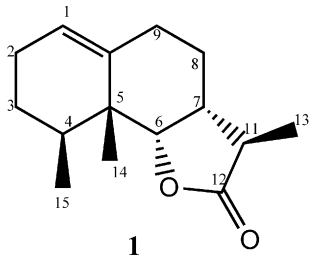

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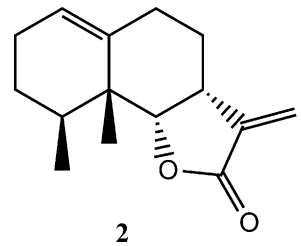

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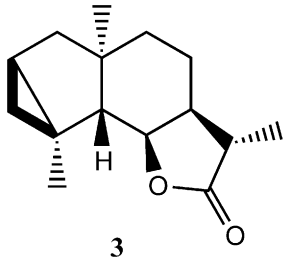

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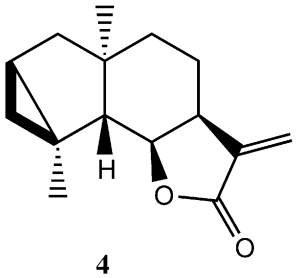

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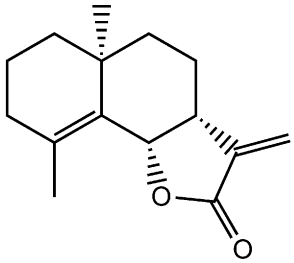

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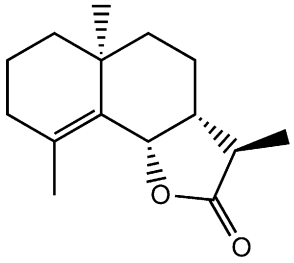

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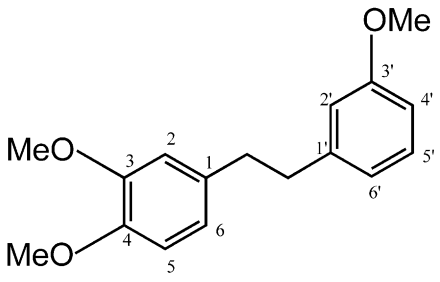

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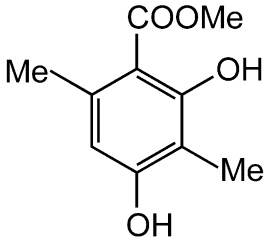

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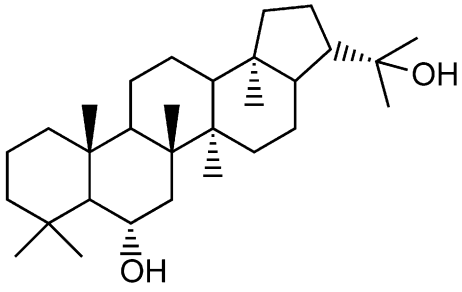

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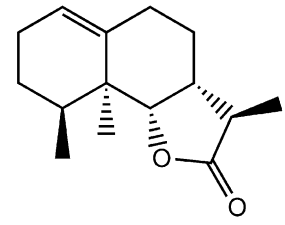
2. Results and discussion

F. brasiliensis is widespread in rain forests of the northwestern mountains of Argentina ("yungas" regions) and, by traditional knowledge, it is known to produce skin contact dermatitis. The air-dried material was extracted with diethyl ether and then methanol. The diethyl ether extract was analyzed by GC–MS to detect the presence of the characteristic mass spectra of either α-methyl or α-methylene γ-lactones. A combination of column chromatography on silica gel, Sephadex LH-20, and preparative HPLC of the ether extract, furnished the crystalline new compound **1**, 5-epidilatanolide A. Its structure was assigned by spectroscopic and X-ray crystallographic analyses. It is a sesquiterpene lactone that showed a molecular ion at m/z 234, and its HR-mass spectrum indicated a molecular formula $C_{15}H_{22}O_2$ accounting for five degrees of unsaturation. The FT–IR spectrum showed a band at 1771 cm^{-1} for a saturated γ-lactone, as well as a C–C double bond absorption (weak) at 1668 cm^{-1}. Accordingly, the ^{13}C NMR spectrum (Table 1) disclosed an ester carbonyl signal at δ



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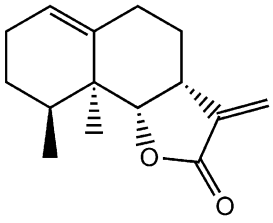

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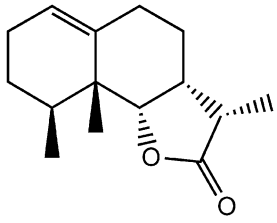

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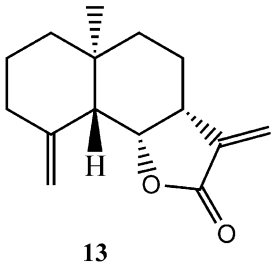

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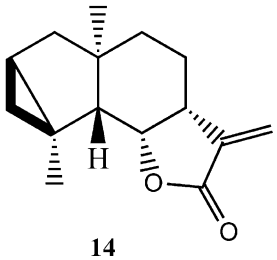

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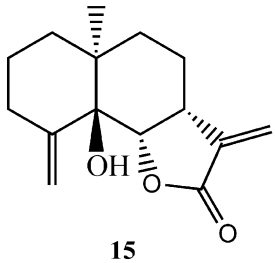

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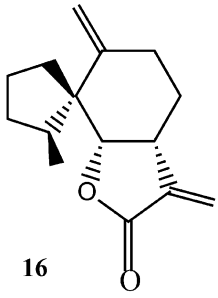

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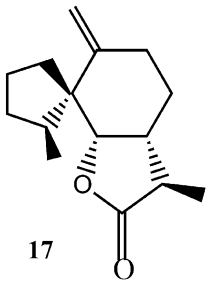

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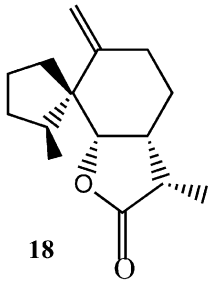

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179.9 and an oxygenated methine carbon at δ 82.8, indicating the presence of a lactone ring. A trisubstituted double bond was evident by the olefinic signals at δ 137.6 and 124.2 for a quaternary and a methine carbon, respectively. In the ^{1}H NMR spectrum (Table 2) of **1**, one tertiary and two secondary methyl signals were present at δ 1.28, 0.97, and 0.91, while the only vinyl proton signal was observed at δ 5.47. The ^{1}H–^{1}H COSY, HMQC and HMBC spectra (Table 3) suggested an eremophilane skeleton for **1**, and confirmed the position of its substituents. The proposed relative stereochemical assignments at C-4, C-5, C-6, C-7, and C-11 were established by the NOESY spectrum (Fig. 1). Ring conformations shown in Fig. 1 are also supported by the observed coupling constants $J_{1,2\beta}=2.5$, $J_{1\text{-}2\alpha}=5$, $J_{6,7}=3.7$, $J_{7,11}\sim 0$, $J_{7,8\alpha}=J_{8\alpha,9\beta}=14$, $J_{8\beta,9\alpha}=4$ and $J_{8\beta\text{-}9\beta}=3$ Hz in the ^{1}H NMR spectrum. Definite proof of the structure of **1** was subsequently obtained from X-ray crystallographic analysis of suitable colorless crystals obtained from a hexane solution. The crystals of **1**

Table 1
^{13}C NMR chemical shifts for compounds **1**, **2**, **3** and **12** (100 MHz, $CDCl_3$ δ values)[a]

C	1	2	3	12
1	124.2	124.4	45.6	124.0
2	25.5	25.4	25.5	23.5
3	26.7	26.6	33.7	25.5
4	32.9	32.6	26.3	32.6
5	39.6	39.4	56.6	39.6
6	82.8	82.5	78.6	83.5
7	40.3	39.3	41.9	38.3
8	28.6	29.3	21.9	29.7
9	30.1	29.4	32.0	32.6
10	137.6	137.0	53.2	137.8
11	45.0	142.6	36.5	42.4
12	179.9	170.4	179.5	179.2
13	13.9	119.2	13.8	9.2
14	17.7	18.0	18.6	17.6
15	15.2	15.2	18.6	15.1

[a] Signals of all compounds were assigned by means of 2D NMR experiments.

Table 2
^{1}H NMR chemical shifts for compounds **1**, **2**, **3** and **12** (400 MHz, $CDCl_3$ δ values)

H	**1**	**2**	**3**	**12**[c]
1a	5.47 *dtbr* (5, 2)	5.50 *dtbr* (5, 2)	1.77 *dd* (13, 7)	5.48 *dtbr* (5, 2)
1b			0.88 *dd* (13, 3.5)	
2a	2.09–2.13[b]	2.05–2.15[b]	1.14 *m*	2.04–2.11[b]
2b	1.91 *dtt* (18, 5, 2.5)	1.94 *dtt* (18, 5, 2.5)		1.92 *dtt* (18, 5, 2.5)
3a	1.42–1.58[b]	1.40–1.50[b]	0.90–0.95[b]	1.40–1.46[b]
3b	1.42–1.58[b]	1.40–1.50[b]	0.34 *t* (4.4)	1.40–1.46[b]
4	2.13–2.18[b]	2.22 (13, 6.5)		2.19 *dq* (15.6, 6.9)
5			0.90–0.95[b]	
6	4.40 *d* (3.7)	4.25 *d* (5)	4.64 *dd* (11.5, 7)	4.18 *d* (3, 6)
7	2.15–2.20[b]	3.09 *ddd* (11, 6, 5)	2.30 *tt* (13,7)	2.50 *dtd* (12.4, 6, 3.6)
8a	1.74 *dddd* (14, 7, 4, 3)	1.81 *ddd* (13.5, 6, 4.5)	1.82 *m*	1.67 *dddd* (12.9, 6, 3.5, 3)
8b	1.21 *qd* (14, 4)	1.35 *dddd* (13.5, 13, 11, 4.5)	1.67 *m*	1.09 *tdd* (12.9, 12.4, 4.4)
9a	2.24 *tbr* (14)	2.32 *ddbr* (14, 13)	1.44 *ddd* (13, 5.5, 2)	2.24 *ddbr* (14, 13)
9b	2.00 *ddd* (14, 4, 3)	2.05 *ddd* (14, 4.5, 4)	1.20–1.35[b]	2.07 *ddd* (14, 4.4, 3)
11	2.31 *q* (7.5)		2.38 *dq* (13, 6.5)	2.78 *dq* (6, 7)
13a	1.28 *d* (7.5)[a]	5.53 *s*	1.19 *d* (6.5)[a]	1.14 *d* (7)[a]
13b		6.07 *s*		
14	0.91 *s*	0.97 *s*	0.98 *s*[a]	1.00 *s*
15	0.97 *d* (7)	0.91 *d* (6.5)	1.26 *s*[a]	0.90 *d* (6.9)

[a] Three proton intensity.
[b] Overlapping signals.
[c] 600 MHz spectrum.

Table 3
HMBC correlations for compounds **1** and **2**

Compound **1**		Compound **2**	
H	C	H	C
1	2, 3, 5, 9	1	3, 5, 9
2a	1, 3, 10	3	1, 2, 4, 5, 15
3	1, 2, 4, 5, 15	6	5, 7, 8, 10, 14
6	4, 5, 7, 8, 10, 11, 14	7	8, 9, 11, 12, 13
8a	6, 7, 9, 10	8a	6, 7, 9, 10
8b	7, 9, 10, 11	8b	7, 9, 11
9a	1, 7, 8, 10	9a	1, 7, 8, 10
11	6, 7, 8, 12, 13	13a	7, 12
13	7, 11, 12	13b	7, 11, 12
14	4, 5, 6, 10	14	4, 5, 6, 10
15	3, 4, 5	15	3, 4, 5

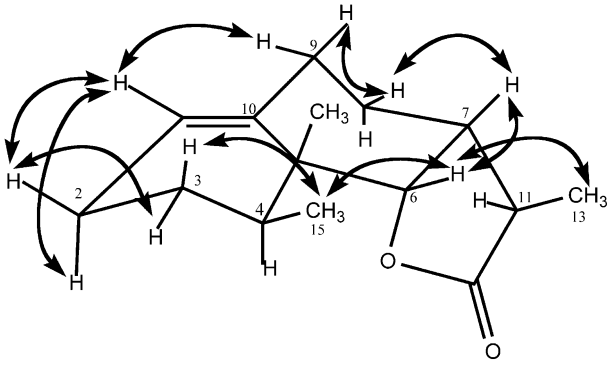

Fig. 1. Partial NOEs observed for compound **1**.

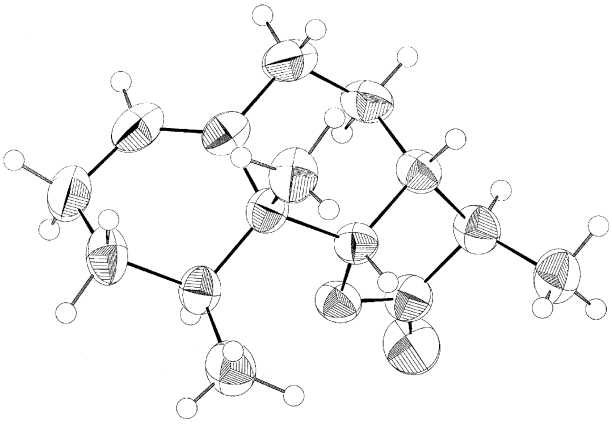

Fig. 2. ORTEP drawing for compound **1**.

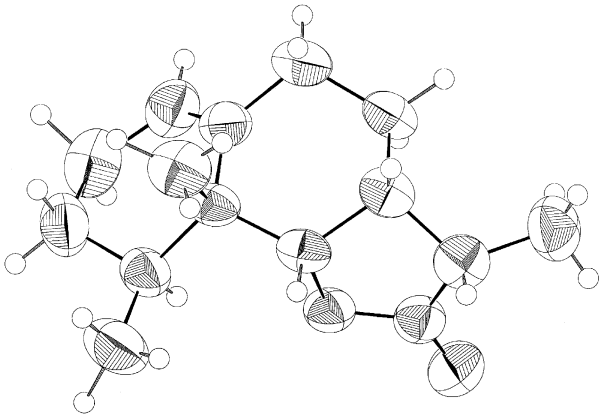

Fig. 3. ORTEP drawing for compound **12**.

are orthorhombic belonging to the space group $P2_12_12_1$. As shown in the ORTEP drawing in Fig. 2, compound **1** possesses an eremophilanolide skeleton and the γ-lactone closed at C-6 and *cis*-annelated to one of the six-membered rings adopts an envelope conformation. The methyl groups at C-5 and C-11 are axially oriented while the Me at C-4 lies in an equatorial conformation. It also confirmed the location of the $\Delta^{1,10}$ double bond and the relative stereostructure of this 5-epidilatanolide, showing that H-6 and H-7 are both on the β face as the Me on C-5, while the lactone ring is oriented on the α side of the molecule. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, and the structure factor table are deposited as supplementary material at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University Crystallographic Laboratory, Tokushima, Japan.

The HR-mass spectrum of compound **2**, 5-epidilatanolide B, indicated a molecular formula $C_{15}H_{20}O_2$. It was obtained as an oil with IR absorptions at 1765 and 1665 cm^{-1}, and ^{13}C NMR signals (Table 1) at δ 170.4 (*s*), 142.6 (*s*), 119.2 (*t*), and 82.5 (*d*), which account for an exomethylene γ-lactone moiety. The ^1H NMR data (Table 2) of **2** suggested the presence of a 6,12-eremophilanolide, closely related to compound **1**. An extensive NMR study, including ^1H, ^{13}C, HMQC, HMBC, and NOESY experiments allowed assignment of structure **2**. Additional evidence was provided by the $NaBH_4$ reduction of **2** that furnished the dihydro-crystalline derivative **12**, an epimer of **1** at C-11. Colorless crystals could be analyzed by X-ray and a stereoscopic view of the molecule is shown in Fig. 3. Absolute configurations of **1** and **2** were established by their CD spectra. The $n\rightarrow\pi^*$ transition of the carbonyl group gives rise to weak absorption bands at around 260 and 215 nm for exomethylene and for saturated γ-lactones, respectively. In addition, Cotton effects have been observed for these lactonic compounds at those wavelengths. Particularly, for the sesquiterpene lactones (+)-frullanolide, **5**, ($\Delta\varepsilon_{265}$ −1.5), dilatanolide A, **10**, ($\Delta\varepsilon_{214}$−4.46), dilatanolide B, **11**, ($\Delta\varepsilon_{262}$−0.30), β-frullanolide, **13**, ($\Delta\varepsilon_{254}$−1.5), brothenolide, **14** ($\Delta\varepsilon_{255}$−1.6), and oxyfrullanolide, **15**, ($\Delta\varepsilon_{259}$−1.52), spirodilatanolides A (**16**) ($\Delta\varepsilon_{265}$−0.51) and spirodilatanolide B (**17**) ($\Delta\varepsilon_{217}$−1.52) isolated from *Frullania* species of liverworts (Asakawa, 1982; Nagashima et al., 1994), absolute configurations were as depicted, based on the negative sign of the Cotton effect. As the sign of the Cotton effect for compounds **1** ($\Delta\varepsilon_{216}$−1.80) and **2** ($\Delta\varepsilon_{255}$−1.69) was negative, we assumed that they belong to the same chiroptical series as **5**, **10**, **11** and **13**–**17**, and consequently the shown absolute configuration was assigned. Noteworthy, the same sign of the Cotton effect is observed for compounds **2**, **11** and **16** and their 11,13- dihydro analogues **1**, **10** and **17**, respectively. The lactones cited above possess *cis*-fusion and α-orientation of the lactone ring. Additionally, in the mentioned dihydro-derivatives, the Me-13 is β-oriented.

The synthetic 5-epidilatanolide **12** differs from the natural compound **1** only in the orientation of the CH_3-13. Its ^1H NMR spectrum (Table 2) showed, as expected, similar features to those of **1**, with little differences. The H-11 signal in the proton spectrum of **12** is

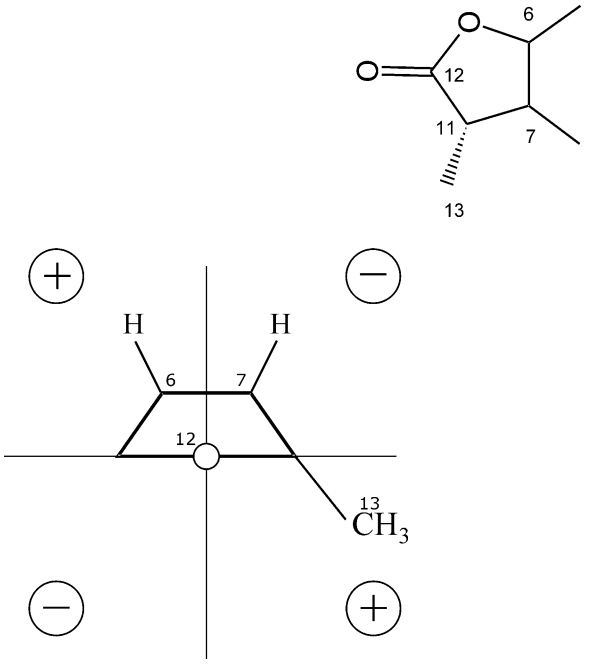

Fig. 4. Octant projection diagram for the partial structure of compound **12**.

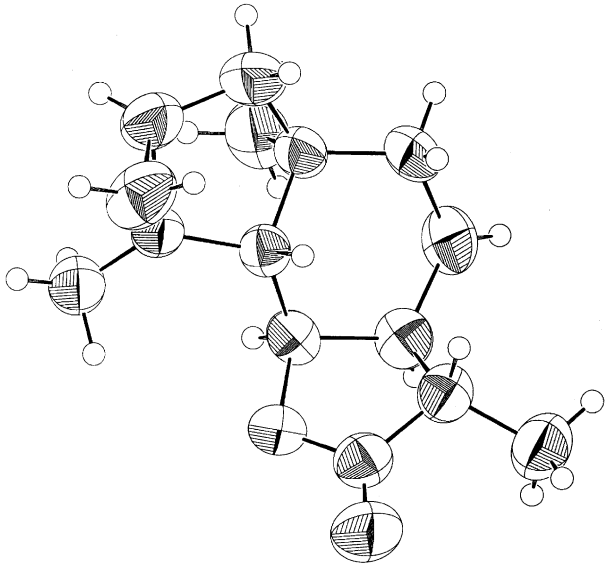

Fig. 5. ORTEP drawing for compound **3**.

0.47 ppm downfield compared with the corresponding H-11 signal of compound **1** (Table 2). In addition, the H-11 signal in the proton spectrum of **12** was a double quartet with $J_{7,11}=6$ Hz and $J_{11,13}=7$ Hz for an α-oriented methyl at C-11, while the H-11 signal for **1** is a quartet suggesting that no coupling occurs between H-7 and H-11 when the methyl group on C-11 is β oriented. In the ^{13}C NMR spectrum of **12** (Table 1) the C-13 signal (δ 9.2) is 4.7 ppm upfield compared with the corresponding C-13 signal for compound **1** (δ 13.9). The described differences in NMR spectral signals for H-11 and C-13 between the C-11 epimeric lactones **1** and **12** resemble the NMR differences for H-11 and C-13 signals previously reported by Nagashima et al. (1994), for the two C-11 epimeric lactones, **17**, isolated from *F. dilatata* and **18**, obtained by reduction of the natural exomethylene γ-lactone **16** also isolated from *F. dilatata*. The CD spectrum of **12** showed a positive Cotton effect ($\Delta\varepsilon_{216}+0.53$) as observed for *cis*-closed β-oriented lactones, such as nepalensolide A (**4**) (Tori et al., 1990), although from a synthetic point of view, compound **12** should display a *cis*-closed α-oriented lactone ring. No reports were found offering an explanation regarding a change in the Cotton effect sign by a change in configuration of C-11 for *cis*-fused γ-lactones. However, the observed positive Cotton effect can be predicted for **12** by the back octant rule (Djerassi, 1960; Moffitt et al., 1961). Due to its pseudoaxial conformation (in accord with the ^1H NMR spectral data, Table 2) the CH_3-13 lies in the lower right (+) octant (Fig. 4), while the remainder of the substituents surrounding the carbonyl chromophore have symmetrical partners and therefore exert low "weight" to the Cotton effect. On the other hand, the contribution of the Me-13 group to the effect should be bigger than that exerted by H-11 which lies in the upper right (-) octant. The predicted net contribution is then positive in compound **12**, which, therefore, changed in the Cotton effect sign in relation to that observed for the exomethylene γ-lactone analogue **2**.

It is strongly suggested that the allergenic properties of *F. brasiliensis* (skin dermatitis detected while collecting the species in the field) are caused by α-methylene butyrolactones, such as compounds **1**–**6**, present in this species (Asakawa, 1982, 1995).

Compound **3** was a solid with an IR absorption at 1770 cm^{-1} and ^{13}C NMR spectroscopic signals at δ 179.5 and 78.6 accounting for a saturated γ-lactone. From the ^1H NMR spectral signals at δ 0.34, 0.88 and 0.92, the presence of a cyclopropane ring was evident. The ^1H and ^{13}C NMR spectra, as well as the HMQC, HMBC and NOESY experiments, suggested that **3** should be nepalensolide B, an eudesmanolide previously isolated from *F. nepalensis* (Tori et al., 1988) and *F. serrata* (Asakawa et al., 1991). When the structure of this compound was previously proposed (Tori et al., 1988), there was a brief report of the NMR spectral data. Full assignment of the ^1H (Table 2) and ^{13}C (Table 1) NMR spectra are presented herein. The colorless isolate could be induced to crystallize from a hexane solution and thus its X-ray analysis was carried out. Crystal data are given in the Experimental. As shown in the corresponding ORTEP drawing in Fig. 5, the *trans*-fusion of the two six member-rings of the eudesmanolide was clear with a *trans* diaxial arrangement for Me-14 and H-5. The *cis*-fused γ-lactone ring is in a conformation that approximates an envelope with C-11 as the flap. The methyl groups attached to the lactone ring, and to C-4, are located in equatorial conformations. The cyclopropane ring lies on the same side of the molecule as the lactone ring while H-6 and H-7 are on the same side as the methyl group at the C-10 position, which is consistent with the results from the NOESY experiment.

The spectroscopic data for compound **4** suggested its close structural relation with **3**, and it was assigned the structure of nepalensolide A, previously isolated from *F. nepalensis* (Tori et al., 1988) and *F. serrata* (Asakawa et al., 1991). Compound **5**, (+)-frullanolide, was the major constituent of this collection of *F. brasiliensis*, the spectral data of which were in good agreement with those of an authentic sample isolated from a Bulgarian collection of *F. dilatata* (Nagashima et al., 1994). Compound **6** (Asakawa et al., 1976) was the dihydro analogue of **5** and was previously isolated from a French collection of *F. dilatata*. Its structure was clearly established by its spectroscopic data.

Regarding the observed configurations at the chiral centers of the previously described sesquiterpene lactones, it is important to point out that the species here described produces four lactones possessing an identical configuration at C-7 (the 5-epidilatanolides **1** and **2**, (+)-frullanolide, **5**, and (+)-dihydrofrullanolide, **6**, and four in which the Me-14 is α-oriented (the nepalensolides **3** and **4**, (+)-frullanolide and (+)-dihydrofrullanolide). Noteworthy, in the eremophilanolides **1** and **2** the Me-14 is β oriented while, in the eudesmanolides **5** and **6**, it is α oriented. With these considerations it is suggested that the new eremophilanolides **1** and **2** may not be derived biosynthetically from **5** and **6**, by migration of Me-14. The mechanism proposed for the bioconversion of eudesmanes into eremophilanes (Hendrickson, 1959; Erdtman and Norin, 1966) requires the same orientation for the angular Me-14 in the eudesmane precursor and in the eremophilane derivative. Our hypothesis states that different cyclases catalyze the biosynthesis of the different lactone types described herein from the ubiquitous precursor farnesyl diphosphate. The occurrence of more than one synthase has already been reported for the biosynthesis of germacrene D enantiomers in *Solidago canadensis* (Schmidt et al., 1999).

Sesquiterpene lactones of *F. brasiliensis* follow the structural patterns for this kind of compounds found in most liverworts (Asakawa, 1995), which are the *cis*-lactone ring closure and scant oxygenation of the remainder of the skeletal carbons, in consequence, making them volatile compounds. This type of sesquiterpenoid is widespread in several genera of the Asteraceae and a few other families of higher plants, but the sesquiterpene lactones of higher plants are, in most cases, highly oxygenated, non-volatile constituents, with *trans*-lactone ring closure being the most frequent (Herz, 1977; Seaman, 1982).

Bibenzyls are widely distributed in liverworts. Hydroxylated and methoxylated bibenzyls have been isolated from a few *Frullania* species (Asakawa, 1995). Our compound **7** (3,3′,4-trimethoxybibenzyl), isolated in trace amounts, is new as a natural product. It was previously prepared by the hydrogenation of 3,3′,4-trimethoxystilbene (Ronlán et al., 1973). It gave a molecular ion peak at *m*/*z* 272 suggesting the molecular formula to be $C_{17}H_{20}O_3$. Its ^1H and ^{13}C NMR spectroscopic features clearly led us to the gross structure. Location of the methoxy groups, as depicted, was achieved by an extensive NMR study. HMQC and HMBC experiments allowed us to assign the ^1H and ^{13}C spectra.

The mass spectrum of the odoriferous compound **8** showed the $[M]^+$ at *m*/*z* 196, in agreement with the molecular formula $C_{10}H_{12}O_4$. Its UV and IR spectra, as well as the only singlets in the ^1H NMR spectrum, suggested the presence of a methyl ester of dihydroxydimethylbenzoic acid, while the strongly hydrogen-bonded hydroxyl group suggested a salicylate. Location of the remaining groups at positions appropriate to a polyketide origin, gave for **8** the structure of atraric acid previously reported from the higher plants *Xylosma velutina* (Cordell et al., 1977) and *Dianella revoluta* (Cooke and Down, 1971). Related compounds have been reported to be cleavage products of natural depsides in lichens (Huneck and Yoshimura, 1996).

Compound **9** was assigned the structure of the hopane-type triterpenoid zeorin (Huneck and Lehn, 1963). It was established by comparison of spectral and other physical data with those of an authentic sample. This is the first report of the presence of zeorin in a *Frullania* collection. Hopanoids are triterpenes widely distributed among bacteria and cyanobacteria, and have been found in a few genera of liverworts (Toyota and Asakawa, 1993; Grammes et al., 1994; Bardón et al. 1999b), lichens (Huneck and Yoshimura, 1996), and ferns (Murakami and Tanaka, 1988). They have also been isolated from sedimentary organic matter of most varied origin and are ubiquitous molecular fossils derived from the cellular constituents of microorganisms (Ourisson et al., 1979).

3. Experimental

3.1. General

TLC was carried out on silica gel precoated glass plates (Kiesel gel 60 F254, Merck) with *n*-hexane–EtOAc (1:1, 2:1 and 4:1). Godin reagent was used for detection. For normal phase CC, silica gel 60 (70–230 μm, Merck) was employed. A mixture of CH_2Cl_2–MeOH (1:1) was used as solvent for CC on Sephadex LH-20. CD spectra were measured in MeOH and $[\alpha]_D$ measurements were carried out in $CHCl_3$. EIMS were measured at 70 eV. Temperature programming of the GC–MS analysis was performed from 50 °C, then 50–280 °C at 5 °C min^{-1} and finally isothermal at 280 °C for 15 min. NMR spectra were recorded in a Jeol ECP-400 spectrometer (400MHz) or a Varian Unity 600 (600 MHz) at room temperature in $CDCl_3$ solutions. For GC–MS: Hewlett Packard, HP 6890 with HP-5MS column, (30 m×0.25 mm i.d.×0.25 μm); MS: Jeol JMS-AX 500; IR: JASCO FT/IR-5300; UV: SHIMADZU UV-160A; Optical Rotation: JASCO DIP-1000 polarimeter; CD: JASCO J-725 spectropolarimeter; mp: YANACO MP. For separation of mixtures a GILSON HPLC and a Beckman Ultrasphere Silica column were used.

3.2. Plant material, extraction and isolation

Frullania brasiliensis Raddi was collected in July 1996 at Cochuna, Tucumán province, Argentina. A voucher specimen (A. Bardón # V) is on deposit at Abt. Systematische Botanik, Albrecht-von-Haller Institut, Göttingen, Germany. The air-dried material (350 g) was extracted with Et_2O for 15 days, followed by MeOH for 1 month, at room temp. Each extract was filtered and the solvents evaporated at red. pres. Small amounts of the Et_2O extract and CC fractions were analyzed by TLC and GC–MS to detect the presence of several types of sesquiterpene lactones, bibenzyls, and phytosterol derivatives. The Et_2O extract (3.25 g) was applied to a silica gel column eluted with a gradient solvent system of *n*-hexane–EtOAc (100:0→0:100) to give 5 fractions (I–V). Fr. III (385 mg) was then subjected to Sephadex LH-20 chromatography, followed by repeated prep. HPLC using *n*-hexane–EtOAc mixtures (3:1 and 4:1) to afford 11 mg of **1**, 26 mg of **2**, 10 mg of **3**, 5 mg of **4**, 104 mg of **5**, 4.5 mg of **6**, 1 mg of **7**, and 3.8 mg of stigmasta-4,22-dien-3-one. After Sephadex LH-20 chromatography, Fr. IV (85 mg) was processed by HPLC with *n*-hexane–EtOAc (7:3) to yield 0.7 mg of **8**, 6.9 mg of sitosterol, and 1 mg of stigmasterol. HPLC of Fr. V (216 mg) using *n*-hexane–EtOAc (7:3) yielded 7.8 mg of **9**. The MeOH extract (402 mg) was applied to silica gel using $CHCl_3$–EtOAc (100:0→0:100) as eluant to give 3 fractions (I–III). HPLC of Fr. I (90 mg) using *n*-hexane–EtOAc (4:1) yielded 6.5 mg of stigmasta-4,22-dien-3-one

and 4.1 mg of stigmasta-4,22-dien-3,6-dione, identified by their spectral features. The remaining fractions furnished unresolved mixtures.

3.3. Compound **1**

Colorless solid; mp 106–107 °C; $[\alpha]_D$ −21.5° ($CHCl_3$; *c* 0.40); HREIMS: found 234.1618 $C_{15}H_{22}O_2$ requires 234.1611; FTIR ν_{max} cm^{-1}: 3027, 1771; ^1H NMR and ^{13}C NMR: Tables 1 and 2; EIMS *m*/*z* (rel. int.): 234 $[M]^+$ (95), 219(37), 206 (20), 191 (11), 177 (39), 161(87), 145 (71), 131 (40), 119 (62), 105 (80), 91 (100), 79 (61), 67 (36), 55 (70); CD: $\Delta\varepsilon_{216}$−1.80 (*c* 5.88×10^{-4}).

3.4. Compound **2**

Colorless oil; $[\alpha]_D$ +50.8° ($CHCl_3$; *c* 0.27); HREIMS: found 232.1458 $C_{15}H_{20}O_2$ requires 232.1463; FTIR ν_{max} cm^{-1}: 3030, 1765, 1665; ^1H NMR and ^{13}C NMR: Tables 1 and 2; EIMS *m*/*z* (rel. int.): 232 $[M]^+$ (71), 217 (39), 204 (20), 189 (12), 175 (37), 163 (32), 145 (35), 131 (34), 119 (57), 105 (73), 91 (100), 77 (62), 65 (36), 53 (62); CD: $\Delta\varepsilon_{255}$−1.69 (*c* 7.76×10^{-4}).

3.5. Compound 7

Colorless oil; EIMS *m*/*z* (rel. int.): 272 $[M]^+$ (29), 151 (100), 107 (6), 91 (5), 78 (4), 65(3); ^1H NMR (600 MHz, $CDCl_3$): δ 7.20 (*t*, *J* = 7.7 Hz, H-5′), 6.79 (*d*, *J* = 8 Hz, H-5), 6.78 (*dt*, *J* = 8, 2 Hz, H-4′), 6.74 (*ddd*, *J* = 8, 2.5, 1.5 Hz, H-6′), 6.73 (*dd*, *J* = 8, 1.9 Hz, H-6), 6.72 (overlapped, H-2′), 6.66 (*d*, *J* = 1.9 Hz, H-2), 3.86, 3.83, 3.78 (*s*, OMe on C-4, C-3 and C-3′, respectively), 2.87 (*sbr*, 4 H); ^{13}C NMR spectrum (150 MHz, $CDCl_3$): δ 159.6 (C-4′), 148.7 (C-3), 147.2 (C-4), 143.4 (C-1′), 134.3 (C-1), 129.2 (C-5′), 120.9 (C-4′), 120.2 (C-6), 114.2 (C-2′), 111.8 (C-2), 111.2 (C-6′), 111.1 (C-5), 55.9 (OMe on C-4), 55.8 (OMe on C-3), 55.1 (OMe on C-3′), 38.2, and 37.4 (2 CH_2).

3.6. X-ray crystallographic analysis of **1**

Compound **1** was recrystallized from *n*-hexane. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer. Data collection: DIP Image plate. Cell refinement: maXus. Data reduction: maXus. The program used to solve the structure: maXus SIR92. $C_{15}H_{22}O_2$, MW = 234.00, orthorhombic, $P2_1$, *a* = 6.280(0) Å, *b* = 9.193(0) Å, *c* = 23.116(0) Å, *V* = 1334.50(0) $Å^3$, *Z* = 4, *D*x = 1230 Mg m^{-3}, *Dm* = 1200 Mg m^{-3}, λ (Cu K_α) = 0.71073 Å, cell parameters from 100 reflections θ = 1-13°, μ = 0.75 mm^{-1}, *T* = 298 K, absorption correction: spherical, θ_{max} = 25.30°, 1248 measured reflections, 1236 independent reflections, 1119 observed reflections, refinement on *F*, *R* = 0.043, *wR* = 0.174, *S* = 1.475, 1119 reflections, 220 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max}$ = 0.6623, $\Delta\rho_{max}$ = 0.26 e $Å^{-3}$, $\Delta\rho_{min}$ = −0.18 e$Å^{-3}$, extinction correction: none.

3.7. X-ray crystallographic analysis of **3**

Compound **3**, mp 126–127 °C, was recrystallized from *n*-hexane. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer. Data collection: DIP Image plate. Cell refinement: maXus. Data reduction: maXus. The program used to solve the structure: maXus SIR92. $C_{15}H_{22}O_2$, MW = 234.00, Monoclinic, $P2_1$, *a* = 9.471(0) Å, *b* = 13.602(0) Å, *c* = 10.453(0) Å, β = 92.023(0)°, *V* = 1334.50(0) $Å^3$, *Z* = 4, λ (Cu K_α) = 0.71073 Å, cell parameters from 100 reflections θ = 1-13°, μ = 0.74 mm^{-1}, *T* = 298 K, absorption correction: none, θ_{max} = 25.30°, 2666 measured reflections, 2659 independent reflections, 1861 observed reflections, refinement on *F*, *R* = 0.067, *wR* = 0.099, S = 2.302, 1861 reflections, 312 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max}$ = 0.2437, $\Delta\rho_{max}$ = 0.34 e $Å^{-3}$, $\Delta\rho_{min}$ = −0.35 e $Å^{-3}$, extinction correction: none.

3.8. $NaBH_4$ reduction of **2**

To a solution of **2** (6 mg) in EtOAc (2 ml), 10 mg of $NaBH_4$ were added and the mixture stirred for 3 h at room temp. Work up as usual gave a residue containing 98% of **12**, a C-11 epimer of **1**. Compound **12** could be induced to crystallize from a solution of *n*-hexane–EtOAc, mp 112–114 °C; $[\alpha]_D$ −24.6°; FTIR ν_{max} cm^{-1}: 1772 (γ-lactone); for ^1H NMR and ^{13}C NMR spectra, see Tables 1 and 2; GC–MS *m*/*z* (rel. int.): 234 $[M]^+$ (91), 219 (37), 206 (14), 191 (10), 177 (37), 161 (91), 145 (72), 131 (44), 119 (62), 105 (79), 91 (100), 79 (61), 67 (36), 55 (75).

3.9. X-ray crystallographic analysis of **12**

Compound **12** was recrystallized from *n*-hexane–EtOAc. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer. Data collection: DIP Image plate. Cell refinement: maXus. Data reduction: maXus. The program used to solve the structure: maXus SIR92. $C_{15}H_{22}O_2$, MW = 234.00, Monoclinic, $P2_1$, *a* = 9.970(0) Å, *b* = 6.401(0) Å, *c* = 10.640(0) Å, β = 90.002(0)°, *V* = 679.00(0) $Å^3$, *Z* = 2, *D*x = 1230 Mg m^{-3}, *Dm* = 1200 Mg m^{-3}, λ (Cu K_α) = 0.71073 Å, cell parameters from 100 reflections θ = 1-13°, μ = 0.74 mm^{-1}, *T* = 298 K, absorption correction: spherical, θ_{max} = 25.30°, 1412 measured reflections, 1409 independent reflections, 1255 observed reflections, refinement on *F*, *R* = 0.047, *wR* = 0.072, *S* = 1.061, 1255 reflections, 219 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max}$ = 0.4002, $\Delta\rho_{max}$ = 0.10 e $Å^{-3}$, $\Delta\rho_{min}$ = −0.20 e $Å^{-3}$, extinction correction: none.

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