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MINOR CONSTITUENTS OF *LIPPIA INTEGRIFOLIA*

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**ABSTRACT.**—Three new sesquiterpenes (**1–3**) possessing the 2,6,6,9-tetramethyltricyclo[5.4.0.0<sup>2,4</sup>]undecane (lippifoliane) skeleton were isolated as minor constituents of *Lippia integrifolia*. Their structures and stereochemistry were elucidated from nmr data including double resonance experiments, COSY, and nOe, and by comparison of their <sup>13</sup>C-nmr spectra with those of lippifoli-1(6)-en-5-one.

Following the studies on the chemical constituents of the aromatic shrub *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae) (**1–5**), we report herein the isolation, structure elucidation, and full spectral analyses of three new substances **1–3** found as minor components in the essential oil of this plant. These tricyclic sesquiterpenes are structurally related to lippifoli-1(6)-en-5-one [**4**], previously isolated as one of the major constituents (**2,3**).

## RESULTS AND DISCUSSION

Compounds **1** and **2** showed [M]<sup>+</sup> at *m/z* 236 in agreement with the molecular formula C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>. The <sup>1</sup>H-nmr spectra of **1** (Table 1) showed H-6 as a doublet (*J*=ca. 1 Hz) due to long-range coupling with H-4, through the carbonyl group at C-5, as found in cyclohexanones (**6**). This coupling was confirmed by double resonance and COSY. These experiments also gave the information to establish the CH<sub>3</sub>(15)-CH(4)-CH<sub>2</sub>(3)-CH<sub>2</sub>(2)-C(1) and CH<sub>2</sub>(8)-CH(9)-CH<sub>2</sub>(11) fragments. In addition, long range couplings between H-8α and Me-13 and between H-11α and Me-14, detected by double resonance, were very useful to assign the tertiary methyl groups.

Although the <sup>1</sup>H-nmr spectra, double resonance, and COSY experiments of **2**

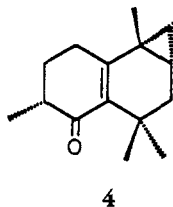
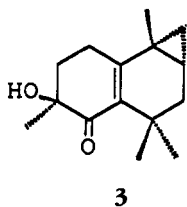
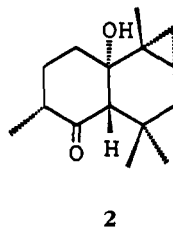
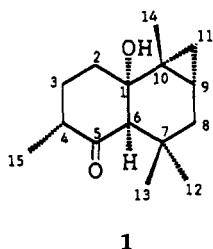


TABLE 1.  $^1\text{H}$ -nmr Data of Compounds **1**–**3**<sup>a</sup>.

Proton	Compound					
	<b>1</b>		<b>2</b>		<b>3</b>	
	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$
H-2 $\alpha$ ...			1.96–2.08 m	1.48–1.70 m	2.25 ddd	1.71–1.99 m
H-2 $\beta$ ...	2.31, 1.84 m	1.90, 1.68 m	1.96–2.08 m	1.72 ddd	2.76 ddd	2.25 m
H-3 $\alpha$ ...	1.96, 1.47 m	1.47, 1.14 m	1.79 m	1.48–1.70 m	2.14 ddd	1.71–1.99 m
H-3 $\beta$ ...			1.96–2.08 m	1.48–1.70 m	1.92 m	1.71–1.99 m
H-4 ...	2.39 m	1.93 m	2.38 dddq	1.92 dddq	—	—
H-6 ...	2.39 d	2.08 d	2.32 brs	1.71 brs	—	—
H-8 $\alpha$ ...	2.06 brdd	2.39 brdd	1.56–1.74 m	1.59 dd	1.17 m	1.01 brdd
H-8 $\beta$ ...	1.59 ddd	1.59 ddd	1.56–1.74 m	1.47 brdd	1.92 m	1.73 dd
H-9 ...	0.86 dddd	0.73 dddd	0.92 m	0.65 dddd	1.13 m	0.79 dddd
H-11 $\alpha$ ...	–0.09 t	0.04 t	1.00 t	0.87 t	0.25 t	–0.04 t
H-11 $\beta$ ...	0.46 dd	0.30 dd	0.24 dd	–0.03 dd	0.91 dd	0.56 dd
Me-12 ...	1.00 s	1.13 s	0.94 brs	1.08 brs	1.09 s	1.23 s
Me-13 ...	1.11 brs	1.10 brs	1.28 s	1.28 s	1.26 brs	1.33 brs
Me-14 ...	1.09 brs	0.92 brs	1.14 brs	1.14 brs	1.24 brs	0.86 brs
Me-15 ...	1.00 d	0.95 d	1.01 d	1.08 d	1.24 d	1.15 brs
H-O ...	1.55 brs	0.41 brs	— <sup>b</sup>	0.57 brs	4.12 brs	4.34 brs

<sup>a</sup>Measured at 300 MHz.  $J$  (Hz) for compound **1**:  $J_{4,6}=1.0$ ,  $J_{4,15}=6.7$ ,  $J_{6,8\beta}=1.8$ ,  $J_{8\alpha,8\beta}=13.8$ ,  $J_{8\alpha,9}=3.0$ ,  $J_{8\alpha,13}=1.5$ ,  $J_{8\beta,9}=9.3$ ,  $J_{9,11\alpha}=4.8$ ,  $J_{9,11\beta}=8.9$ ,  $J_{11\alpha,11\beta}=4.8$ . For compound **2**:  $J_{2\alpha,2\beta}=12.7$ ,  $J_{2\beta,3\alpha}=11.6$ ,  $J_{2\beta,3\beta}=5.5$ ,  $J_{3\alpha,4}=13.0$ ,  $J_{3\beta,4}=6.5$ ,  $J_{4,15}=6.4$ ,  $J_{4,6}=1.0$ ,  $J_{8\alpha,8\beta}=14.3$ ,  $J_{8\alpha,9}=2.0$ ,  $J_{8\beta,9}=5.8$ ,  $J_{9,11\alpha}=5.7$ ,  $J_{9,11\beta}=9.1$ ,  $J_{11\alpha,11\beta}=4.8$ . For compound **3**:  $J_{2\alpha,2\beta}=18.7$ ,  $J_{2\alpha,3\alpha}=5.7$ ,  $J_{2\alpha,3\beta}=11.4$ ,  $J_{2\beta,3\alpha}=1.7$ ,  $J_{2\beta,3\beta}=6.3$ ,  $J_{3\alpha,3\beta}=13.2$ ,  $J_{3\beta,15}=0.5$ ,  $J_{8\alpha,8\beta}=14.2$ ,  $J_{8\alpha,9}=J_{8\beta,9}=J_{9,11\alpha}=4.2$ ,  $J_{9,11\beta}=8.0$ ,  $J_{11\alpha,11\beta}=3.9$ .

<sup>b</sup>Not observed.

yielded basically the same connectivity pattern as in **1**, we observed an important change in the coupling constants of the  $\text{CH}_2(8)\text{-CH}(9)$  fragment due to a change in the conformation of the six-membered ring. An analysis of the vicinal coupling constants by using a generalized Karplus-type equation (7,8), as recently done for *trans*-humul-(9*E*)-ene-2,6-dione (**5**), allows us to draw the conformation of **1** and **2** as shown in Figure 1.

The  $^{13}\text{C}$ -nmr spectra in combination with DEPT analysis of **1** and **2** (Table 2) provided conclusive evidence to support that both hydrates (**1** and **2**) of **4** have the

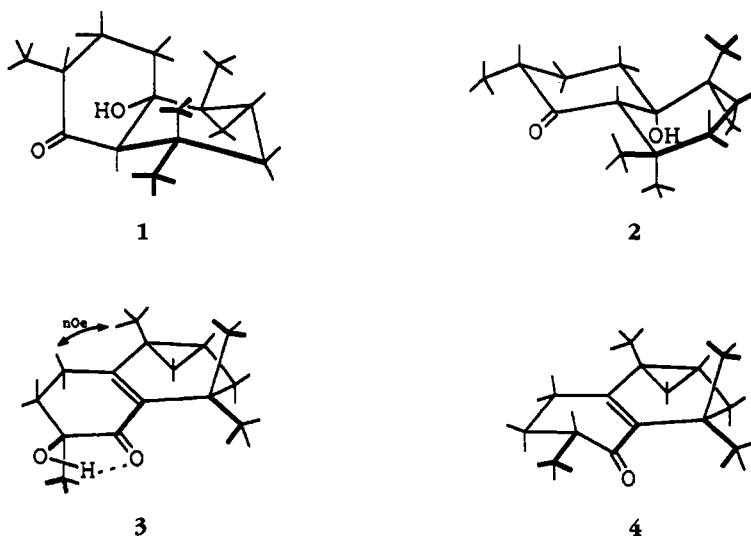
FIGURE 1. Preferred conformation of **1**–**4** in solution.

TABLE 2.  $^{13}\text{C}$ -nmr Chemical Shifts and Multiplicities<sup>a</sup> of Compounds 1–4.

Carbon	Compound					
	1		2		3	4 <sup>b</sup>
	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$	$\text{C}_6\text{D}_6$	$\text{CDCl}_3$
C-1 .....	77.6 s	76.8	78.7 s	77.9	157.9 s	160.2
C-2 .....	33.7 t	34.2	38.8 t	38.7	25.5 t	28.7
C-3 .....	27.6 t	27.7	31.9 t	31.6	36.7 t	30.2
C-4 .....	44.9 d	44.9	46.4 d	46.1	72.5 s	42.4
C-5 .....	213.7 s	212.7	212.0 s	209.8	203.4 s	201.4
C-6 .....	62.6 d	62.8	63.7 d	63.3	138.4 s	140.0
C-7 .....	30.9 s	31.1	30.6 s	31.0	33.9 s	33.5
C-8 .....	37.5 t	38.0	39.4 t	39.7	43.5 t	43.5
C-9 .....	19.1 d	19.5	22.6 d	22.6	19.3 d	19.1
C-10 .....	23.7 s	23.9	24.7 s	24.6	19.5 s	20.4
C-11 .....	20.9 t	20.9	15.7 t	16.0	27.4 t	26.3
C-12 .....	28.6 q	28.9	34.0 q	34.0	28.1 q	28.9
C-13 .....	31.7 q	31.9	25.5 q	25.5	28.4 q	27.5
C-14 .....	22.3 q	22.4	24.4 q	24.4	22.7 q	22.8
C-15 .....	14.8 q	15.0	14.4 q	15.0	23.7 q	16.7

<sup>a</sup>From DEPT analysis measured at 75.4 MHz.<sup>b</sup>Values for this compound are from Catalán *et al.* (10).

tertiary hydroxyl group at C-1 and not at C-6, because each substance showed a signal for a quaternary carbon around 77 ppm. If the hydroxyl group was at C-6, a higher value could be expected. Furthermore, each compound (**1** and **2**) showed a signal for a CH around 63 ppm. This high chemical shift value can be explained by the influence of the carbonyl group at C-5 and the methyl group at C-7, as observed in natural products having a similar fragment (**9**).

Since **1** and **2** have two additional chiral centers when compared to **4**, we looked for spectral evidence to establish their relative stereochemistry. Thus, when the chemical shifts of C-11 and C-14 in **4** were compared with the corresponding values of **1** and **2** (Table 2), we observed an important change for C-11 but not for C-14. Given that C-11 is located at the  $\alpha$  side and C-14 at the  $\beta$  side in the three substances, the hydroxyl group at C-1 in **1** and **2** must be  $\alpha$ -oriented.

On the other hand, the stereochemistry at C-6 in **1** and **2** could be assigned when we observed a considerable nOe effect (ca. 25%) between H-6 and H-11 $\alpha$  in compound **1**. Therefore, compound **1** corresponds to the 1,6-*cis*-stereoisomer and **2** to the 1,6-*trans*-stereoisomer.

Compound **3** showed  $[\text{M}]^+$  at  $m/z$  234 as required for  $\text{C}_{15}\text{H}_{22}\text{O}_2$ . While its structure followed straightforwardly when comparing  $^{13}\text{C}$ -nmr chemical shifts with those of **4** (Table 2), the stereochemistry of the chiral center at C-4 required further consideration. The  $^1\text{H}$ -nmr spectrum of **3** in  $\text{CDCl}_3$  showed Me-15 as a doublet with a very small coupling constant (ca. 0.5 Hz), confirmed by careful irradiation, due to a long-range coupling with one of the protons at C-3. This fact is in favor of an axial orientation of both the Me-15 and the coupled H-3. Furthermore, we observed a nOe effect (11%) between Me-14 and one of the protons at C-2. Since the relative stereochemistry of Me-14 is  $\beta$ , the enhanced H-2 is H-2 $\beta$ . In addition, the vicinal coupling constants of this proton revealed its equatorial orientation. By consideration of these arguments, the only self-consistent stereostructure for **3** is as drawn in Figure 1, in which Me-15 has an  $\alpha$  orientation.

The  $^{13}\text{C}$ -nmr assignments for the protonated carbons of **1–3** are based on DEPT experiments, on literature data (10), and on residual  $^{13}\text{C}$ - $^1\text{H}$  coupling constants extracted from partially coupled spectra measured by setting the  $^1\text{H}$  decoupler frequency on the TMS signal. Since heteronuclear residual couplings mainly depend on the frequency difference between the resonance frequency and the decoupler frequency (11–13), these distinctions are possible once the  $^1\text{H}$  signals are assigned.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Hplc separations were performed on Waters equipment (M45 pump, U6K injector with 2 ml loop and R-401 differential refractometer), using (A) a Beckman ODS column (5 $\mu$ , 10 $\times$ 250 mm) and (B) a Maxsil Phenomenex 10 C8 column (10 $\times$ 500 mm). Retention times were measured from the injection solvent peak. Nmr spectra were measured on a Varian Associates XL-300GS spectrometer from solutions containing TMS as the internal reference. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and mass spectra were obtained at 70 eV on a Hewlett Packard HP-5988A spectrometer. Melting points are uncorrected. Ir spectra were recorded on a Perkin-Elmer 16 FPC FT spectrophotometer.

**COLLECTION, EXTRACTION, AND ISOLATION.**—Air-dried aerial parts of *L. integrifolia* were purchased from a local market and authenticated by botanist A. Slanis from Miguel Lillo Institute, Tucumán, Argentina, where a sample (voucher no. C Catalán 580) is deposited. The essential oil was obtained by steam distillation. From 3.1 kg of aerial parts, 18.3 g of oil (0.59%) was obtained. The oil was chromatographed on a Si gel (230–400 mesh) column using hexane with increasing amounts of  $\text{Et}_2\text{O}$  (2–40%); 142 fractions were collected and monitored by tlc. Fractions 62–67, containing spathulenol (**1**) as the major component, were combined (703 mg) and a portion (260 mg) processed by hplc using column B, eluting with  $\text{MeOH-H}_2\text{O}$  (2:1) at 2 ml/min. Two fractions were collected. The first contained a peak with Rt 17.1 min and the second a peak with Rt 19.0 min. The first fraction contained compound **1**, which was purified by hplc rechromatography on column A with  $\text{MeOH-H}_2\text{O}$  (2:1) at 2.5 ml/min to give **1** (6.2 mg, Rt 23.5 min). The second fraction contained **2** and **3**, which were separated by rechromatography on column A, as above, to yield **2** (8.2 mg, Rt 22 min) and **3** (11.3 mg, Rt 31 min).

**1,6-cis-Lippifolian-1 $\alpha$ -ol-5-one [1].**—White solid: mp 62–65°;  $[\alpha]_{\text{D}}^{25}$  0,  $[\alpha]_{\text{D}}^{25}$  0,  $[\alpha]_{\text{D}}^{25}$  0,  $[\alpha]_{\text{D}}^{25}$  +23°,  $[\alpha]_{\text{D}}^{25}$  +234° ( $c$ =0.04,  $\text{CHCl}_3$ ); ir ( $\text{CHCl}_3$ )  $\nu$  max 3590, 3380, 1710, 1460, 1375, 1235, 1070  $\text{cm}^{-1}$ ; ms  $m/z$  (rel. int.)  $[\text{M}]^+$  236 (18), 179 (58), 137 (23), 109 (23), 83 (63), 78 (63), 41 (100);  $^1\text{H}$  nmr see Table 1;  $^{13}\text{C}$  nmr see Table 2.

**1,6-trans-Lippifolian-1 $\alpha$ -ol-5-one [2].**—White solid: mp 73–76°;  $[\alpha]_{\text{D}}^{25}$  -57,  $[\alpha]_{\text{D}}^{25}$  -57,  $[\alpha]_{\text{D}}^{25}$  -64,  $[\alpha]_{\text{D}}^{25}$  -96,  $[\alpha]_{\text{D}}^{25}$  -89 ( $c$ =0.11,  $\text{CHCl}_3$ ); ir ( $\text{CHCl}_3$ )  $\nu$  max 3600, 3380, 1710, 1460, 1365, 1215  $\text{cm}^{-1}$ ; ms  $m/z$  (rel. int.)  $[\text{M}]^+$  236 (45), 208 (61), 179 (100), 152 (15), 126 (42), 109 (35), 83 (60), 67 (26), 41 (95);  $^1\text{H}$  nmr see Table 1;  $^{13}\text{C}$  nmr see Table 2.

**Lippifoli-1(6)-en-4 $\beta$ -ol-5-one [3].**—White solid: 49–52;  $[\alpha]_{\text{D}}^{25}$  -81,  $[\alpha]_{\text{D}}^{25}$  -83,  $[\alpha]_{\text{D}}^{25}$  -103,  $[\alpha]_{\text{D}}^{25}$  -241,  $[\alpha]_{\text{D}}^{25}$  -699 ( $c$ =0.61,  $\text{CHCl}_3$ ); ir ( $\text{CHCl}_3$ )  $\nu$  max 3590, 3460, 1665, 1595, 1436, 1385, 1365, 1235, 1170  $\text{cm}^{-1}$ ; uv ( $\text{MeOH}$ )  $\lambda$  max (log  $\epsilon$ ) 256 (4.04); ms  $m/z$  (rel. int.)  $[\text{M}]^+$  234 (22), 206 (20), 176 (100), 161 (65), 133 (55), 105 (38), 91 (37), 77 (28), 43 (86);  $^1\text{H}$  nmr see Table 1;  $^{13}\text{C}$  nmr see Table 2.

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