

TRACE CONSTITUENTS OF *LIPPIA INTEGRIFOLIA*

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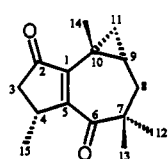
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ABSTRACT.—Three new sesquiterpenes, together with *S*-(+)-*trans*-nerolidol, were isolated from the essential oil of *Lippia integrifolia*. Their structures were elucidated by nmr spectroscopy as 2 β ,4 β ,9 α -2,6,6,9-tetramethyltricyclo[6.3.0.0^{2,4}]undec-1(8)-ene-7,11-dione [**1**], 1 β ,2 α ,8 β -2,6,10,10-tetramethylbicyclo[6.3.0]undec-5-en-2-ol (3 α -hydroxy-6-asteriscene) [**2**], and 1 α ,7 β ,9 α -1-hydroxy-3,6,6,9-tetramethylbicyclo[5.4.0]undec-3-en-8-one [**3**].

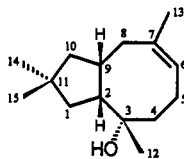
The aerial parts of the aromatic shrub *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae) are used in the traditional medicine of some regions of central and northern Argentina as a diuretic, emmenagogue, stomachic, and nervine agent (1). Previous chemical studies on the essential oil of this abundant species revealed the presence of sesquiterpenes with uncommon carbon skeletons (2–7). These facts encouraged us to explore further its trace constituents, whereby three new substances (**1–3**) were found, together with the known sesquiterpene (*S*)-(+)-*trans*-nerolidol (8,9). Although IUPAC names are given in some parts of this paper, the discussion uses conventional numbering for the natural products, as represented in **1–3**.

The structure of compound **1** was determined from its ¹H- and ¹³C-nmr data (Tables 1 and 2) in C₆D₆ and CDCl₃. The ¹H-nmr spectrum in CDCl₃ showed signals for the CH₃(15)-CH(4)-CH₂(3)-C(2)=O fragment. The coupling constants $J_{3\alpha,4}$ and $J_{3\beta,4}$ showed values of 2.3

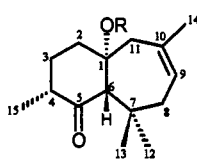
and 6.7 Hz, respectively, which are typical of cyclopentenones (10). Furthermore, the same spectrum showed a spin-spin system very similar to that found in lippifoli-1(16)-en-5-one (4) for the CH₂(8)-CH(9)-C(10)-CH₂(11) fragment. This system was confirmed by spin simulation (11). In addition, the ¹³C-nmr spectrum of **1** (Table 2), assigned from a partially coupled spectrum (7) and from a HETCOR experiment, showed a large chemical shift difference for the quaternary carbon bearing the *gem*-dimethyl group C-7 (47.0 ppm) as compared to lippifoli-1(16)-en-5-one (33.5 ppm) (12). This is due to the presence of the carbonyl group at C-6 in **1**. The uv spectrum showed a λ max at 257 nm, characteristic of a O=C-C=C-C=O moiety and the ir spectrum exhibited the corresponding bands at 1712, 1671, and 1650 cm⁻¹. The eims showed a [M]⁺ at *m/z* 232 in agreement with a molecular formula of C₁₅H₂₀O₂. The stereochemistry of **1**, identical to substances isolated previously from the same species (2–7), was sup-



1

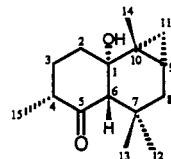


2



3 R=H

5 R=CONHCOCCL₃



4

TABLE 1. ¹H-Nmr Data of Compounds 1, 3, and 5.^a

Proton	Compound					
	1		3		5	Δ(5-3)
	C ₆ D ₆	CDCl ₃	C ₆ D ₆	CDCl ₃	CDCl ₃	CDCl ₃
H-2α ..	—	—	1.39-1.54 m	1.91 br dd	3.00 br dd	1.09
H-2β ..	—	—	1.39-1.54 m	1.74 m	1.95 m	0.21
H-3α ..	1.63 dd	2.06 dd	1.39-1.54 m	1.74 m	1.39 m	-0.35
H-3β ..	2.18 dd	2.71 dd	1.69-1.81 m	1.88 dddd	2.00 dddd	0.12
H-4 ...	3.07 ddq	3.35 ddq	1.89 m	2.37 br ddq	2.42 br ddq	0.05
H-6 ...	—	—	2.01 br s	2.45 br s	2.41 br s	-0.04
H-8α ..	0.94 br dd	1.24 br dd	1.59 dd	1.77 dd	1.86 br dd	0.09
H-8β ..	1.60 dd	2.02 dd	1.91 br dd	2.11 br dd	2.11 br dd	0.00
H-9 ...	0.68 dddd	1.06 dddd	5.54 tsext.	5.71 tsext.	5.68 m	-0.03
H-11α .	0.39 t	0.51 t	1.86 dd	2.13 dd	3.09 dd	0.96
H-11β .	0.77 dd	0.95 dd	2.45 br dd	2.80 br dd	2.85 br dd	0.05
Me-12 .	0.88 s	1.06 s	1.49 s	1.35 s	1.29 s	-0.06
Me-13 .	1.21 s	1.35 s	1.03 s	0.98 s	1.00 s	0.02
Me-14 .	1.14 s	1.20 s	1.67 td	1.80 td	1.75 td	-0.05
Me-15 .	0.66 d	1.04 d	1.02 d	0.99 d	1.00 d	0.01

^aMeasured at 300 MHz in ppm from TMS as the internal reference. *J* (Hz) for compound 1: $J_{3\alpha,3\beta}=19.0$, $J_{3\alpha,4}=2.4$, $J_{3\beta,4}=6.8$, $J_{4,15}=7.1$, $J_{8\alpha,8\beta}=14.8$, $J_{8\alpha,9}=10.9$, $J_{8\beta,9}=5.2$, $J_{9,11\alpha}=4.5$, $J_{9,11\beta}=4.4$. For compound 3 and 5 $J_{2\alpha,2\beta}=14.3$, $J_{2\alpha,3\alpha}=4.4$, $J_{2\alpha,3\beta}=2.0$, $J_{2\beta,3\beta}=5.1$, $J_{3\alpha,3\beta}=11.0$, $J_{3\alpha,4}=13.0$, $J_{3\beta,4}=5.5$, $J_{4,15}=6.0$, $J_{8\alpha,8\beta}=14.2$, $J_{8\alpha,9}=8.3$, $J_{8\beta,9}=6.4$, $J_{9,11\beta}=1.7$, $J_{9,11\alpha}=1.7$, $J_{9,14}=1.7$, $J_{11\alpha,11\beta}=14.2$

ported by a NOESY experiment carried out in C₆D₆ in which correlations between H-3α and H-11α, H-3α and Me-12, H-11α and Me-15, Me-12 and Me-

13, and Me-12 and Me-15 were observed.

The eims of compound 2 showed a [M-18]⁺ at *m/z* 204. The structure was deduced from the combined evaluation

TABLE 2. ¹³C-Nmr Chemical Shifts and Multiplicities of Compounds 1-3.^a

Carbon	Compound				
	1		2		3
	C ₆ D ₆	CDCl ₃	C ₆ D ₆	CDCl ₃	C ₆ D ₆
C-1	166.4 s	167.6 s	44.2 t	43.9 t	78.5 s
C-2	210.0 s	211.4 s	51.1 d	50.8 d	42.0 t ^b
C-3	43.1 t	43.3 t	74.1 s	74.7 s	31.6 t
C-4	32.8 d	32.8 d	42.7 t	42.2 t	46.3 d
C-5	147.4 s	147.3 s	25.0 t	24.5 t	210.6 s
C-6	207.1 s	209.2 s	125.8 d	125.1 d	69.1 d
C-7	46.8 s	47.0 s	133.4 s	133.8 s	30.2 s
C-8	41.2 t	41.2 t	34.4 t	34.2 t	43.6 t ^b
C-9	20.2 d	20.2 d	41.4 d	41.2 d	126.8 d
C-10	16.8 s	16.4 s	50.1 t	49.8 t	135.8 s
C-11	22.1 t	21.5 t	33.7 s	33.7 s	47.5 t
C-12	27.9 q	27.9 q	23.9 q	23.8 q	32.6 q
C-13	23.7 q	23.7 q ^b	27.9 q	27.6 q	26.3 q
C-14	23.7 q	23.6 q ^b	31.9 q	31.8 q	22.8 q
C-15	19.0 q	19.2 q	31.9 q	31.8 q	15.1 q

^aMeasured at 75.4 MHz in ppm from TMS as the internal reference.

^bAssignments may be interchanged.

of ^1H -nmr (Table 3), COSY, HETCOR, and FLOCK (13) experiments. The COSY diagram allowed the location of the molecular segments constituted by protonated carbons C-1-C-2-C-9-C-8-C-10 and C-4-C-5-C-6, and the FLOCK diagram (C-H long range couplings) completed the structure by showing strong correlations between C-1 and H-14, C-1 and H-15, C-10 and H-14, C-10 and H-15, and C-3 and H-4. In addition, irradiation of the vinylic proton at C-6 confirmed the four-bond coupling between this proton and H-8 α . These data, in combination with the ^{13}C -nmr chemical shifts (Table 2) were consistent with a molecule having the 2,6,10,10-tetramethylbicyclo[6.3.0]undecane skeleton, which has been named asteriscane (14). The relative stereochemistry at C-2 and C-9 in **2** is *cis* from a comparison of the coupling constant value between H-2 and H-9 (11.0 Hz) with that of

asteriscanolide ($J_{2,9}=11.4$ Hz), which has been studied by X-ray diffraction (14). The C-3 epimer of **2**, obtained as an intermediate of a biomimetic sequence for the transformation of humulene into 7-protoilludyl cation derivatives (15), turned out to be helpful in establishing the stereochemistry of **2** at C-3. Thus, molecular modelling calculations (6,7,16) of **2** and its C-3 epimer, together with the analysis of the vicinal coupling constants of **2** by means of a Karplus-type equation (17,18), revealed that the eight-membered rings of both substances adopt a conformation close to a chair C_5 (19). The methyl group at C-3 in **2** remains pseudo-axial, while in the C-3 epimer, it remains pseudo-equatorial. These results are consistent with the observed chemical shifts of these methyl groups as measured in CCl_4 (**2**, 1.06 ppm vs. its C-3 epimer, 1.15 ppm), and therefore with the stereochemistry at C-3 for both substances.

The eims of compound **3** showed a $[\text{M}-18]^+$ fragment peak at m/z 218. The ^1H -nmr spectrum (Table 1) in CDCl_3 showed signals for a vinylic proton at 5.71 ppm (H-9) as a triple sextet and a vinylic methyl group at 1.80 ppm (Me-14) as a triple doublet. These signals indicated the presence of a $\text{CH}_2(11)\text{-C}(10)(\text{CH}_3)=\text{CH}(9)\text{-CH}_2(8)$ fragment. Furthermore, the signals for both methylene groups (H-8 α , H-8 β , H-11 α , and H-11 β), assigned by double-resonance experiments, showed no additional vicinal couplings, except for one of the protons of the methylene group attached to the vinylic methyne (H-8 β), which showed a long-range coupling with the signal of Me-12. Therefore, the fragment corresponded to $\text{C}(1)\text{-CH}_2(11)\text{-C}(10)(\text{CH}_3)=\text{CH}(9)\text{-CH}_2(8)\text{-C}(7)(\text{CH}_3)_2$. The signals for the $\text{CH}_3(15)\text{-CH}(4)\text{-CH}_2(3)\text{-CH}_2(2)\text{-C}(1)$ fragment could be recognized easily in the ^1H -nmr spectrum measured in C_6D_6 . The signal for H-4 showed the same multiplicity and coupling constant values as in the previously described analogue **4** (7). Furthermore, a long-range coupling constant

TABLE 3. ^1H -Nmr Data of Compound **2**.^a

Proton	2	
	C_6D_6	CDCl_3
H-1 α	1.35 dd	1.40 dd
H-1 β	1.63 dd	1.62 dd
H-2	2.06 ddd	2.09 ddd
H-4 α	1.90 ddd	2.02 ddd
H-4 β	1.49 br ddd	1.58 br ddd
H-5 α	1.97 m	2.06 m
H-5 β	2.22 m	2.27 m
H-6	5.39 dddq	5.42 dddq
H-8 α	2.31 br dd	2.40 br dd
H-8 β	1.80 dd	1.91 dd
H-9	1.70 m	1.86 m
H-10 α	1.24 t	1.28 t
H-10 β	1.43 dd	1.55 dd
Me-12	1.02 s	1.14 s
Me-13	1.70 q	1.72 q
Me-14	1.01 s ^b	1.01 s ^b
Me-15	1.00 s ^b	1.02 s ^b

^aMeasured at 300 MHz in ppm from TMS as the internal reference. J (Hz): $J_{1\alpha,1\beta}=12.7$, $J_{1\alpha,2}=12.0$, $J_{1\beta,2}=7.3$, $J_{2,9}=11.0$, $J_{4\alpha,4\beta}=14.3$, $J_{4\alpha,5\alpha}=2.7$, $J_{4\alpha,5\beta}=12.0$, $J_{4\beta,5\alpha}=7.2$, $J_{4\beta,5\beta}=3.2$, $J_{5\alpha,6}=7.3$, $J_{5\beta,6}=5.9$, $J_{5\alpha,13}=1.3$, $J_{5\beta,13}=1.3$, $J_{6,8\alpha}=1.3$, $J_{6,13}=1.3$, $J_{8\alpha,8\beta}=14.0$, $J_{8\alpha,9}=5.1$, $J_{8\beta,9}=4.9$, $J_{9,10\alpha}=12.3$, $J_{9,10\beta}=6.8$, $J_{10\alpha,10\beta}=12.0$.

^bAssignments may be interchanged.

between H-4 and H-6, through the C-5 carbonyl group, was also observed, as was the case for **4** (7). The ^{13}C -nmr spectrum of **3** (Table 2) was also in agreement with the proposed structure.

The relative stereochemistry of **3** was deduced when its ^1H -nmr data were compared with those of derivative **5** (Table 1), obtained by addition of a drop of trichloroacetyl isocyanate to a CDCl_3 solution of **3**. It is known (20) that when the derivative is formed, the signals attributable to protons vicinal to hydroxyl groups experience large shifts ($\Delta\delta$ 0.2–1.2 ppm) when they are syn to the oxygen atom and small shifts ($\Delta\delta$ 0.0–0.2 ppm) when they are anti. The values found in this case for the α -protons (Table 1) revealed a trans relationship between the oxygen atom at C-1 and the hydrogen atom at C-6. The coupling constants of H-3 α with H-4 (13.0 Hz) and H-3 β with H-4 (5.5 Hz) indicated the axial orientation of H-4. Therefore, assuming a chair conformation for the six-membered ring, the methyl group at C-4 has an equatorial orientation and it is cis with respect to the oxygen atom at C-1. In the substances previously isolated from *L. integrifolia* (2–7), the humulene pattern can be recognized easily, but in the natural product **3** it seems that its biogenesis may involve a methyl migration from C-11 to C-10, as has been observed in some related sesquiterpenes, in which a cyclopropane ring is present at some point of the biogenetic pathway (21). Alternatively, the rearrangement may occur via a non-classical carbonium ion involving C-1, C-9, C-10, and C-11.

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 μm , 10×250 mm) and (b) a Maxsil Phenomenex 10 C_8 column (10×500 mm). Retention times were measured from the injection solvent peak. The 300 MHz ^1H -nmr spectra were measured on a Varian Associates XL-300GS spectrometer from solutions containing TMS as the

internal reference and the 90 MHz ^1H -nmr spectrum of **2** was measured on a Varian Associates EM-390 spectrometer in CCl_4 solutions. ^{13}C -Nmr spectra were determined with a Varian Associates XL-300GS spectrometer. 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. Mps are reported uncorrected. Ir spectra were recorded on a Perkin-Elmer 16 FPC Ft spectrophotometer.

PLANT MATERIAL.—As reported previously (6,7).

EXTRACTION AND ISOLATION.—Aerial parts of *L. integrifolia* were processed and chromatographed as described previously (6,7). A portion of fractions 62–67 (260 mg) was processed by hplc using column (b), eluting with $\text{MeOH-H}_2\text{O}$ (2:1) at 2 ml/min. A peak at R_f 12.5 min was collected and purified by rechromatography on the same column to afford **1** (12.7 mg). A peak at R_f 17.1 min was collected and rechromatographed on column (a) to afford **3** (3.2 mg) at 27.5 min. Peaks at 44 and 55 min were purified by rechromatography on column a to yield **2** (8.7 mg) and *S*-(+)-*trans*-nerolidol (13.4 mg), respectively.

2 β , 4 β , 9 α -2, 6, 6, 9-Tetramethyltricyclo[6.3.0.0 2,4]undec-1(8)-en-7,11-dione [1].—Colorless oil; $[\alpha]_{589} +127^\circ$, $[\alpha]_{578} +134^\circ$, $[\alpha]_{546} +159^\circ$, $[\alpha]_{436} +345^\circ$, $[\alpha]_{365} +1071^\circ$ ($c=0.69$, CHCl_3); ir (film) ν max 3060, 1712, 1671, 1650, 1455, 1380, 1235, 1145, 1036 cm^{-1} ; uv (MeOH), λ max (log ϵ) 257 nm (4.08); cims (CH_4) m/z $[\text{M}+\text{H}]^+$ 233 (100), 205 (24), 191 (13), 177 (12), 161 (9), 148 (13), 135 (16), 111 (19); eims m/z $[\text{M}]^+$ 232 (26), 204 (8), 191 (16), 161 (67), 148 (100), 133 (32), 106 (43), 91 (40), 77 (42); ^1H -nmr data, see Table 1; ^{13}C -nmr data, see Table 2.

1 β , 2 α , 8 β -2, 6, 10, 10-Tetramethylbicyclo[6.3.0]undec-5-en-2-ol [2].—Colorless oil; $[\alpha]_{589} +5^\circ$, $[\alpha]_{578} +6^\circ$, $[\alpha]_{546} +7^\circ$, $[\alpha]_{436} +11^\circ$, $[\alpha]_{365} +14^\circ$ ($c=1.84$, CHCl_3); ir (film) ν max 3400, 1645, 1465, 1380, 1372, 1112 cm^{-1} ; eims m/z $[\text{M}-\text{H}_2\text{O}]^+$ 204 (18), 189 (8), 139 (57), 121 (12), 95 (22), 81 (24), 55 (26); ^1H nmr (300 MHz, CDCl_3 and C_6D_6), see Table 3; ^1H nmr (90 MHz, CCl_4) δ 5.43 (1H, m, H-6), 1.72 (3H, br s, Me-13), 1.06 (3H, s, Me-12), 1.00 ppm (6H, Me-14 and Me-15); ^{13}C -nmr data, see Table 2.

1 α , 7 β , 9 α -1-Hydroxy-3, 6, 6, 9-tetramethylbicyclo[5.4.0]undec-3-en-8-one [3].—White solid; mp 56–59 $^\circ$; $[\alpha]_{589} -25^\circ$, $[\alpha]_{578} -25^\circ$, $[\alpha]_{546} -28^\circ$, $[\alpha]_{436} -49^\circ$, $[\alpha]_{365} -74^\circ$ ($c=0.29$, CHCl_3); ir (CHCl_3), ν max 3592, 3420, 3032, 1712, 1456, 1378, 1240 cm^{-1} ; eims m/z $[\text{M}-\text{H}_2\text{O}]^+$ 218 (3), 167 (18), 139 (11), 127 (25), 110 (100) 83 (57), 55 (38); ^1H -nmr data, see Table 1; ^{13}C -nmr data, see Table 2.

S-(+)-trans-Nerolidol.—Colorless oil; $[\alpha]_{589}$

+13° ($c=1.0$, CHCl_3), lit. (9) $[\alpha]_{589} +15.5^\circ$. The ^{13}C -nmr data of this substance were identical to those reported (8).

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