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## STRUCTURE AND CONFORMATION OF A HUMULENEDIONE FROM *LIPPIA INTEGRIFOLIA*

CÉSAR A.N. CATALÁN,\* MARINA E.P. DE LAMPASONA, INÉS J.S. DE FENIK,

*Instituto de Química Orgánica, Facultad de Bioquímica, Química y Farmacia,  
Universidad Nacional de Tucumán, Ayacucho 491, S.M. de Tucumán 4000, Argentina*

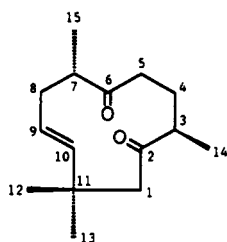
CARLOS M. CERDA-GARCÍA-ROJAS, AND PEDRO JOSEPH-NATHAN\*

*Departamento de Química del Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional,  
Apartado 14-740, México, D.F., 07000 México*

**ABSTRACT.**—A novel diketone having the humulene skeleton was isolated from the essential oils of *Lippia integrifolia*. Its structure was established by spectroscopic studies, and its conformation followed from nOe effects in combination with the H-C-C-H dihedral angles calculated from <sup>1</sup>H-nmr coupling constants.

The aromatic shrub *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae) has been a rich source of unusual sesquiterpenoids. The ketones integrifolian-1,5-dione (1,2), lippifoliol(6)-en-5-one (1), and 4,5-*seco*-african-4,5-dione (3) have been isolated from its essential oils, although  $\alpha$ -humulene,  $\alpha$ -himachalene,  $\beta$ -caryophyllene, and spathulenol are the major components (1,4). This work describes the isolation, structural elucidation, and conformation in solution of a new eleven-membered ring substance, humulenedione [1].

The conformational analysis, in solution, of carbocyclic substances possessing large membered rings has not been a common subject, because their <sup>1</sup>H-nmr spectra frequently present extensive signal overlapping and strong coupling effects (5). Fortunately, the two carbonyl groups in humulenedione [1] cause shifts of several proton signals, thus giving more informative spectra.



1

Humulenedione [1] is found as a minor component in those fractions where integrifolian-1,5-dione and spathulenol are the main constituents and was isolated by reversed-phase hplc using an octadecylsilane column. Cims of 1 shows a quasi-molecular ion at  $m/z$  237  $[M+1]^+$ , which together with elemental analysis pointed to the formula  $C_{15}H_{24}O_2$ . The ir spectrum displays absorptions at 3020 and 986  $cm^{-1}$  for a trans-disubstituted double bond and a strong absorption at 1707  $cm^{-1}$  for a saturated carbonyl function. The uv spectrum shows no significant absorptions between 210 and 360 nm, while evaluation of <sup>1</sup>H-nmr spectra obtained in  $CDCl_3$  and  $C_6D_6$  (see Table 1) indicated a humulene derivative.

Although there are important chemical shift differences between the <sup>1</sup>H-nmr spectra measured in  $CDCl_3$  and  $C_6D_6$  (Table 1) due to the known aromatic-solvent-induced shift effect (6), the vicinal <sup>1</sup>H-<sup>1</sup>H coupling constants remain similar in both

TABLE 1.  $^1\text{H}$ -nmr Data of Humulenedione [1].<sup>a</sup>

Proton	$\text{CDCl}_3$	$\text{C}_6\text{D}_6$
$\text{H}_a$ -1 .....	2.60 br d	2.16
$\text{H}_b$ -1 .....	1.91 dd	1.66
H-3 .....	2.68 dddq	2.64
$\text{H}_a$ -4 .....	1.67 dddd	1.76-1.87
$\text{H}_b$ -4 .....	1.71 dddd	1.76-1.87
$\text{H}_a$ -5 .....	2.48 ddd	2.35
$\text{H}_b$ -5 .....	2.21 ddd	1.76-1.87
H-7 .....	2.64 ddq	2.09
$\text{H}_a$ -8 .....	2.05 ddd	2.00
$\text{H}_b$ -8 .....	2.18 dddd	1.76-1.87
H-9 .....	5.29 ddd	5.02
H-10 .....	5.34 dd	5.06
Me-12 .....	1.16 s	1.19 s
Me-13 .....	1.01 s	0.74 s
Me-14 .....	1.01 d	0.84 d
Me-15 .....	1.00 d	0.89 d

<sup>a</sup>Measured at 300 MHz.  $J$  (Hz,  $\text{CDCl}_3$ ): 1a,1b=11.7; 1b,3=1.2; 3,4a=4.0; 3,4b=10.0; 3,14=7.2; 4a,4b=14.5; 4a,5a=11.7; 4a,5b=3.5; 4b,5a=3.6; 4b,5b=4.0; 5a,5b=19.2; 7,8a=12.0; 7,8b=4.0; 7,15=6.3; 8a,8b=12.6; 8a,9=10.2; 8b,9=3.9; 8b,10=1.2; 9,10=15.7.

solvents. Therefore, in each case the preferred conformation should essentially be the same. The multiplicity and coupling constant values of the vinylic proton signals confirm the trans relationship and C-9/C-10 position of the double bond. Double resonance and COSY experiments performed in both solvents allowed assignment of the  $^1\text{H}$ -nmr signals. The  $W$  couplings between  $\text{H}_a$ -1 and Me-12 and between  $\text{H}_b$ -1 and H-3 combined with  $n\text{Oe}$  between Me-12 and Me-14 (11% in  $\text{C}_6\text{D}_6$ ), support the conformation given in Figure 1 for the C-11-C-1-C-2-C-3-C-14 fragment of the eleven-membered ring. On the other hand, an  $n\text{Oe}$  between H-7 and Me-14 (5% in  $\text{C}_6\text{D}_6$ ) is consistent with the relative stereochemistry of **1**, in which the two secondary methyl groups are trans. The  $n\text{Oe}$  effect is also in agreement with the conformation depicted in Figure 1, in which both H-7 and Me-14 remain pseudo-axial. The coupling constant values of the protons of the C-7-C-8-C-9 and C-3-C-4-C-5 fragments give complementary information useful in predicting the preferred conformation in solution. These values enabled us to calculate dihedral angles by using a generalized Karplus-type

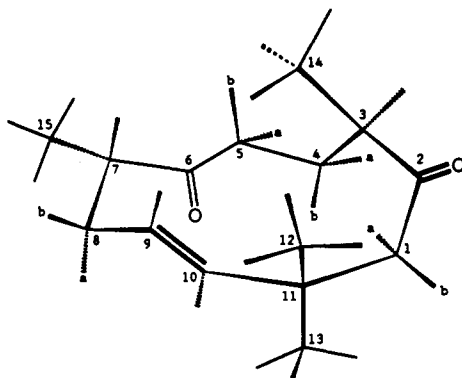


FIGURE 1. Preferred conformation of humulenedione [1] in solution.

TABLE 2. Calculated *H-C-C-H* Dihedral Angles for Humulenedione [1].

Protons	Angle (degrees)
H-3-H <sub>a</sub> -4 .....	+55
H-3-H <sub>b</sub> -4 .....	+152
H <sub>a</sub> -4-H <sub>a</sub> -5 .....	+10
H <sub>a</sub> -4-H <sub>b</sub> -5 .....	-119
H <sub>b</sub> -4-H <sub>a</sub> -5 .....	-119
H <sub>b</sub> -4-H <sub>b</sub> -5 .....	+122
H-7-H <sub>a</sub> -8 .....	+169
H-7-H <sub>b</sub> -8 .....	+55

equation, which in addition considers the coupling constants' dependence on the electronegativity and the stereochemistry of the atoms near to the coupled protons (7,8). The values provided by these calculations (Table 2), together with the information gained using a Dreiding model, show that the conformation depicted in Figure 1 is the most likely possibility. Coupling constants for H-3-H<sub>a</sub>-4, H-3-H<sub>b</sub>-4, H-7-H<sub>a</sub>-8, and H-7-H<sub>b</sub>-8 were measured in the H-3 and H-7 signals during irradiation of the secondary methyl groups, while coupling constants between protons of the C-4-C-5 fragment were ascertained after spin-spin simulation using the LAOCN3 program (9).

In order to obtain the C-C-C-C torsion angles of humulenedione [1] for comparative purposes, a molecular model was generated by using MMX force field calculations as implemented in the PCMODEL program. [Available from Serena Software, this program operates with MMX which is a derived version of the MM2 program developed by N.L. Allinger (QCPE 395), University of Georgia.] Since the MM2 energy surface of cycloundecane contains some 40 conformers (10), our calculations were restricted to generate the energetically minimized conformer corresponding to the experimental <sup>1</sup>H-nmr data. The results are shown in Figure 1, and C-C-C-C torsion angles are summarized in Table 3.

The combined results obtained by spin-spin simulation, nOe measurements, and dihedral angle evaluations and from the MMX model provide a self consistent picture of the molecular conformation.

TABLE 3. Comparison Between the C-C-C-C Torsion Angles (deg) of Humulene bis (AgNO<sub>3</sub>) Adduct (A) and Humulenedione [1].

Atoms	A <sup>a</sup>	1 <sup>b</sup>
C-1-C-2-C-3-C-4 .....	+172	-28
C-2-C-3-C-4-C-5 .....	-108	+148
C-3-C-4-C-5-C-6 .....	+35	-106
C-4-C-5-C-6-C-7 .....	+77	+107
C-5-C-6-C-7-C-8 .....	-161	-123
C-6-C-7-C-8-C-9 .....	+95	+68
C-7-C-8-C-9-C-10 .....	-94	-89
C-8-C-9-C-10-C-11 .....	+155	+179
C-9-C-10-C-11-C-1 .....	-101	-120
C-10-C-11-C-1-C-2 .....	+54	+79
C-11-C-1-C-2-C-3 .....	-126	-77

<sup>a</sup>Calculated from X-ray atomic coordinates (13).

<sup>b</sup>Taken from data of the structure drawn in Figure 1, which was obtained by using a molecular modeling program.

In previous work on conformational analysis of humulene derivatives, the bis(AgNO<sub>3</sub>) adduct of humulene (11–13), diepoxyhumulene (14), and triepoxyhumulene (15) were evaluated after X-ray diffraction studies, showing a common conformation for their macrocycle. Moreover, the study of zerumbone 2,4-dinitrophenylhydrazone by the same technique (16) discloses, for its eleven-membered ring, a conformation that differs in the region where the phenylhydrazone group is located.

On comparing the results obtained in this work with those of the previous reports, a certain correspondence is found between the conformation in solution of **1** and that of humulene bis(AgNO<sub>3</sub>) adduct, except for the C-3–C-4–C-5 fragment. This is shown by the C-C-C-C torsion angles of both substances, listed in Table 3. The torsion angles of humulene bis(AgNO<sub>3</sub>) adduct were calculated from the published X-ray atomic coordinates and cell dimensions (13).

In addition, the specific rotation of humulenedione [**1**] is high ( $[\alpha]_{589} + 201^\circ$ ) due to the presence of an inherently dissymmetric chromophore (17).

Finally, the protonated carbon signals in the <sup>13</sup>C-nmr spectrum of **1** (Table 4) were assigned according to their multiplicity and differences between the values of the C-H residual coupling constants extracted from a partially coupled spectrum, obtained by setting the <sup>1</sup>H decoupler frequency at the TMS signal. The distinction among signals of the same multiplicity was done in light of residual coupling constants values, since they mainly depend on the difference between the resonance frequency and the decoupler frequency (18,19). The latter experiment can often be used instead of <sup>13</sup>C/<sup>1</sup>H chemical shift correlation experiments when low quantities of sample are available (20).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc separations were performed on a Waters instrument (M45 pump, U6K injector with 2 ml loop and R-401 differential refractometer). Nmr spectra were measured on a Varian Associates XL-300GS spectrometer with solutions containing TMS as the internal reference. The ir spectrum was recorded on a Perkin-Elmer 983 spectrophotometer; optical rotation was measured on a Perkin-Elmer 241 polarimeter; and the mass spectrum was obtained on a Hewlett Packard HP-5988A spectrometer. Solid state torsion angles were calculated from literature data (13) using the Data General Nova 4 and Tektronix 4105 computer system linked to a Nicolet R3m single crystal X-ray diffractometer.

COLLECTION, EXTRACTION, AND ISOLATION.—Air-dried aerial parts of *L. integrifolia* were purchased

TABLE 4. <sup>13</sup>C-nmr Data of Humulenedione [**1**].<sup>a</sup>

Carbon	CDCl <sub>3</sub>	C <sub>6</sub> D <sub>6</sub>
C-1 .....	52.5 t	52.3
C-2 .....	214.4 s	212.4
C-3 .....	45.9 d	46.0
C-4 .....	25.2 t	25.8
C-5 .....	42.9 t	42.9
C-6 .....	216.2 s	214.1
C-7 .....	44.7 d	44.5
C-8 .....	40.2 t	40.5
C-9 .....	124.5 d	124.8
C-10 .....	139.1 d	139.3
C-11 .....	37.1 s	37.0
C-12 .....	23.3 q	23.6
C-13 .....	31.8 q	31.7
C-14 .....	18.2 q	18.3
C-15 .....	16.5 q	16.8

<sup>a</sup>Measured at 75.4 MHz.

from a local market and authenticated by botanists of the Miguel Lillo Institute, Tucumán, 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 (mainly leaves), 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 Et<sub>2</sub>O (2–40%); 142 fractions were collected and monitored by tlc. Fractions 62–67, containing mainly spathulenol and integrifolian-1,5-dione as the major constituents, were combined (703 mg) and further purified by hplc on a Phenomenex Maxsil 10 C8 column (10 μm, 10 × 500 mm), with MeOH-H<sub>2</sub>O (2:1) at 2 ml/min. The peak having a retention time of 17 min was collected preparatively and was shown by <sup>1</sup>H nmr to be a 1:3 mixture of **1** and integrifolian-1,5-dione. This mixture was then separated on a Phenomenex Ultremex C18 column (5 μm, 10 × 250 mm) with MeOH-H<sub>2</sub>O (2:1) at 2.5 ml/min to give 15 mg of **1** (Rt 20 min) and 49 mg of integrifolian-1,5-dione (Rt 17 min).

*trans-Humul-(9E)-ene-2,6-dione*.—Colorless oil: ir (film)  $\nu$  max 3020, 1707, 1455, 1406, 1375, 1367, 1288, 1241, 1176, 1150, 1080, 1053, 1030, 986 cm<sup>-1</sup>; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +201°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +215°, [ $\alpha$ ]<sub>D</sub><sup>30</sup> +256°, [ $\alpha$ ]<sub>D</sub><sup>35</sup> +534°, [ $\alpha$ ]<sub>D</sub><sup>36</sup> +1199° ( $c=0.36$ , CHCl<sub>3</sub>); cims (CH<sub>4</sub>)  $m/z$  (rel. int.) [M+1]<sup>+</sup> 237 (59), 219 (81), 181 (100), 165 (49), 163 (15), 127 (12), 111 (13), 109 (21), 95 (14); <sup>1</sup>H nmr see Table 1; <sup>13</sup>C nmr see Table 4. *Anal.* calcd for C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>: C 76.23, H 10.24; found C 76.15, H 10.35%.

MOLECULAR MODELING OF THE PREFERRED CONFORMATION OF **1**.—Humulenedione [**1**] in a conformation close to that deduced from <sup>1</sup>H-nmr data was manually input using the Structure Input Mode of the program PCMODEL (1.0) and then minimized with the MMX-M routine on a HP Vectra ES/12 PC. The structure readily converged to the energetic minimum represented in Figure 1 (27.9 kcal). The C-C-C-C torsion angles were obtained using the QUERY routine of the Display Mode.

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