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REVISION OF THE STRUCTURE OF AN ARISTOLANE SESQUITERPENE ALDEHYDE ISOLATED FROM THE ROOT OF *PLECTRANTHUS HEREROENSIS* AND *ARISTOLOCHIA DEBILIS*

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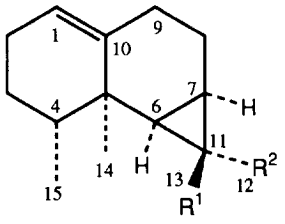
Key Word Index—*Plectranthus hereroensis*; Labiatae; *Aristolochia debilis*; Aristolochiaceae; sesquiterpene; aristolane; 1(10)-aristolen-13-al; structure revision; antimicrobial activity.

Abstract—From the acetone extract of the root of *Plectranthus hereroensis* an aristolane sesquiterpene aldehyde has been isolated. This substance was identical to a compound previously isolated from the root of *Aristolochia debilis* to which the structure of 1(10)-aristolen-12-al was attributed. Now, extensive NMR spectroscopic studies have established that the structure of this substance must be amended to 1(10)-aristolen-13-al. This sesquiterpene showed moderate antimicrobial activity.

INTRODUCTION

In a previous communication [1] we reported the isolation of some abietane diterpenes possessing antimicrobial activity from the acetone extract of the root of *Plectranthus hereroensis*. Now, a study of the less polar chromatographic fractions of the same extract have allowed the isolation of a substance whose physical (mp) and spectroscopic (IR, ^{1}H and ^{13}C NMR and mass spectral) data were identical to those reported [2] for an aristolane sesquiterpene aldehyde previously found in the root of *Aristolochia debilis*. The structure attributed to this substance,§ 1(10)-aristolen-12-al (**1**) [2], must be amended to 1(10)-aristolen-13-al (**2**) on the basis of extensive NOE experiments reported below.



	R^1	R^2
1	Me	CHO
2	CHO	Me

RESULTS AND DISCUSSION

The complete and unambiguous assignment of the proton and carbon resonances of **2** is summarized in Table 1. These data were in agreement with the ^{1}H–^{1}H COSY and HMQC spectra of **2** collected in Table 2. These assignments are almost identical to those reported previously [2], except for the resonances of C-2 and C-3, which must be reversed (see Tables 1 and 2), and for the proton at δ2.43, previously attributed to one of the C-8 methylene protons [2] and now assigned to the H-9α proton. Apart from the observed connectivities (Table 2), this last reassignment was rigorously supported by double resonance experiments, because an allylic coupling (J = 2.1 Hz) observed between the proton at δ2.43 and the C-1 olefinic proton (δ5.35) disappeared when the signal of the H-9α or H-1 protons was irradiated. Moreover, irradiation at δ1.75 (H-7α proton) caused modifications only in the signals at δ1.48 (H-6α), 2.17 (H-8α) and 1.98 (H-8β), thus confirming that the proton appearing at δ2.43 was not attached to the C-8 methylene carbon.

Exhaustive NOE experiments (Table 3) on **2** allowed the assignment of both protons at the C-8 and C-9 methylene groups and provided conclusive proof on the *endo*-configuration of the aldehyde group, which must be placed at C-13 (**2**) instead of the C-12 *exo* position

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§For aristolanes we use the numbering system proposed by Connolly and Hill [3], which differs from that used in ref. [2].

Table 1. ^1H and ^{13}C NMR spectral data of **2***

H			C	
1	5.35 *quint*	($J_{1,2}$ = 4.4)	1	122.1 *d*†
2α	1.98 *m*	($J_{1,2'}$ = 4.9)	2	25.4 *t*
2β	1.98 *m*	($J_{1,9\alpha}$ = 2.1)	3	26.9 *t*
3α	1.45 *m*	($J_{6\alpha,7\alpha}$ = 9.1)	4	40.4 *d*
3β	1.45 *m*	($J_{7\alpha,8\alpha}$ = 9.8)	5	36.7 *s*
4β	1.36 *m*	($J_{7\alpha,8\beta}$ = 3.1)	6	44.8 *d*
6α	1.48 *d*	($J_{8\alpha,8\beta}$ = 14.2)	7	30.8 *d*
7α	1.75 *ddd*	($J_{8\alpha,9\alpha}$ = 8.6)[a]	8	20.9 *t*
8α	2.17 *dddd*	($J_{8\alpha,9\beta}$ = 6.8)[a]	9	28.9 *t*
8β	1.98 *m*	($J_{15,4\beta}$ = 6.5)	10	142.2 *s*
9α	2.43 *m*		11	35.2 *s*
9β	1.98 *m*		12	19.7 *q*
Me-12	1.13 *s*		13	205.0 *d*
13	9.58 *s*		14	22.5 *q*
Me-14	1.19 *s*		15	15.9 *q*
Me-15	0.94 *d*			

*At 500 MHz (^1H) and 125.7 MHz (^{13}C), both in $CDCl_3$ solution. Chemical shifts are relative to the residual $CHCl_3$ (δ7.25 for the ^1H NMR spectrum) and to the solvent (δ_{CDCl_3} 77.00 for the ^{13}C NMR spectrum). All these assignments were in agreement with double resonance and NOE experiments and the HMQC spectrum (see discussion and Tables 2 and 3).

†Multiplicities were determined from the HMQC spectrum (Table 2).

[a]Interchangeable assignments.

previously proposed (**1**) [2]. Irradiation at δ9.58 caused positive NOE enhancement in the signals of the H-1, H-4β, H-8β and Me-12 protons, but not in those corresponding to the cyclopropane (H-6α and H-7α, Table 3), thus establishing that the aldehyde group possesses an *endo*-configuration. On the contrary, irradiation at δ1.13 (Me-12, geminal to the aldehyde group) produced positive NOE enhancement only in the cyclopropane protons (H-6α and H-7α) and in the aldehyde hydrogen (H-13); consequently Me-12 has an *exo*-configuration. Moreover, irradiation at δ2.17 (H-8α) caused positive NOE enhancement in the H-7α, H-8β, H-9α and H-9β protons and a negative NOE enhancement in the signal of the aldehyde proton (H-13) as a consequence of the transference of the NOE through the H-8β proton. The remaining NOE experiments shown in Table 3 further supported the relative stereochemistry depicted in **2** for this aristolane sesquiterpene (e.g., the assignment of the signal at δ2.43 to the H-9α proton, which showed a positive NOE with the Me-14 group).

It is reasonable to assume that in **2** there is a preferred rotamer for the *endo*-aldehyde group owing to the electrostatic repulsion between the C-1/C-10 olefinic double bond and the oxygen atom of the aldehyde. This preferred rotamer, with the aldehyde hydrogen close to the olefinic bond (*endo*-conformation), precludes the *W*-coupling of the aldehyde proton (sharp singlet signal) with the H-6α and H-7α cyclopropane protons, thus explaining the mistake in the structure **1** previously attributed to this sesquiterpenoid [2].

From all the above data it is evident that the substance previously isolated from *Aristolochia debilis* [2] and now found in *Plectranthus hereroensis* possesses the structure depicted in **2**, with the aldehyde group in an *endo*-configuration (C-13).

1(10)-Aristolen-13-al (**2**) showed moderate antimicrobial activity against *Staphylococcus aureus* by the bioautography method [4, 5] (see Experimental).

EXPERIMENTAL

Mp: uncorr. For details on the extraction of *Plectranthus hereroensis* see ref. [1].

Isolation of 1(10)-*aristolen*-13-*al* (**2**). The residue of the least polar chromatographic frs of the acetone extract of the root of *P. hereoensis* (frs eluted with petrol–EtOAc, 19:1, 105 mg) was subjected to CC (silica gel Merck No. 7734, deactivated with 15% H_2O, 40 g)

Table 2. ^1H–^1H COSY and HMQC correlations in **2**

H (δ)	^1H–^1H COSY Correlated with δ (proton)	HMQC Correlated with δ (carbon)
1 (5.35)	1.98 (2α,2β), 2.43 (9α)*†	122.1 (1)
2α,2β (1.98)‡	1.45 (3α,3β), 5.35 (1)	25.4 (2)†
3α,3β (1.45)‡	1.98 (2α,2β), 1.36 (4β)	26.9 (3)†
4β (1.36)	1.45 (3α,3β), 0.94 (Me-15)	40.4 (4)
6α (1.48)	1.75 (7α)	44.8 (6)
7α (1.75)	1.48 (6α), 1.98 (8β)†, 2.17 (8α)	30.8 (7)
8α (2.17)	1.75 (7α), 1.98 (8β,9β), 2.43 (9α)†	20.9 (8)
9α (2.43)	1.98 (8β,9β), 2.17 (8α), 5.35 (1)*	28.9 (9)
Me-12 (1.13)	9.58 (13)*	19.7 (12)
13 (9.58)	1.13 (Me-12)*	205.0 (13)
Me-15 (0.94)	1.36 (4β)	15.9 (15)

*Long-range coupling.

†Reassigned signals with respect to those reported in ref. [2].

‡Interchangeable assignments in ref. [2].

Table 3. NOE experiments on **2***

Irradiation at δ (proton)	Observed NOE enhancement†												
	H-1	H-3α	H-4β	H-6α	H-7α	H-8α	H-8β	H-9α	H-9β	Me-12	H-13	Me-14	Me-15
1.48 (H-6α)	0	0	0		(+ + +)	0	0	0	0	(+)	0	(+)	(+ +)
1.75 (H-7α)	0	0	0	(+ + +)		(+ +)	(+)	0	0	(+ +)	0	0	0
2.17 (H-8α)	0	0	0	0	(+ +)		(+ + +)	(+ +)	(+ +)	0	(−)	0	0
1.13 (Me-12)	0	0	0	(+)	(+)	0	0	0	0		(+)	0	0
9.58 (H-13)	(+)	0	(+ + +)	0	0	0	(+ + +)	0	0	(+)		0	(+ +)
1.19 (Me-14)	0	(+ +)	0	(+)	0	0	0	(+)	0	0	0		0
0.94 (Me-15)	0	(+ + +)	(+ + +)	(+)	0	0	0	0	0	0	(+)	‡	

*Measured at 500 MHz, in $CDCl_3$ solution, by the FT difference method.

†(+), (+ +) and (+ + +) denote weak (0.1–1%), medium (1.1–3%) and strong (> 3%) positive NOE enhancements, respectively. (−) denotes a medium negative NOE enhancement. Zero indicates NOE enhancement not observed.

‡Not measured.

eluting with petrol–EtOAc (49:1). This CC provided 25 mg of impure **2**, which was purified on prep. TLC (silica gel plates, petrol–EtOAc, 49:1, as eluent). Compound **2** (14 mg) crystallized on cooling, mp 65–67°; $[\alpha]_D^{20}$ − 15.4° ($CHCl_3$; *c* 0.078). IR ν_{max}^{KBr} cm^{-1}: 3020, 2960, 2930, 2880, 2770, 1680, 1460, 1435, 1380, 1310, 1195, 1130, 1060, 1025, 990, 925, 835, 750, 730. ^1H and ^{13}C NMR: Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): 218 $[M]^+$ (12), 217 (2), 203 (40), 189 (10), 185 (12), 176 (100), 161 (43), 147 (70), 146 (64), 119 (48), 118 (90), 105 (55), 91 (60), 77 (23), 55 (8). $C_{15}H_{22}O$: M_r 218. Identical in all respects with the previously described compound [2].

The antimicrobial activity of **2** against *Staphylococcus aureus* was assayed by bioautography [4, 5], showing moderate inhibition zones (~ 30% of inhibition).

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REFERENCES

1. Batista, O., Duarte, A., Nascimento, J., Simões, F., de la Torre, M. C. and Rodríguez, B. (1994) *J. Nat. Prod.* **57**, 858.
2. Rücker, G., Mayer, R., Breitmaier, E., Will, G., Kirfel, A. and El Kordy, M. (1984) *Phytochemistry* **23**, 1647.
3. Connolly, J. D. and Hill, R. A. (1991) *Dictionary of Terpenoids*, Vol. 1, p. XXII. Chapman & Hall, Cambridge.
4. Slusarenko, A. J., Longland, A. C. and Whitehead, I. M. (1989) *Bot. Helv.* **99/2**, 203.
5. Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M. and Frenk, E. (1991) *Phytochem. Anal.* **2**, 199.