Phytochemistry, Vol. 27, No. 1, pp. 221–224, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

CANDELABRONE, A NEW ABIETANE DITERPENE FROM THE LEAVES OF *SALVIA CANDELABRUM*

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(*Revised received* 22 *May* 1987)

Key Word Index—*Salvia candelabrum*; Labiatae; diterpenoid diketone; candelabrone; 11,12,14-trihydroxy-8,11,13-abietatriene-3,7-dione.

Abstract—The new diterpenoid candelabrone, isolated from leaves of *Salvia candelabrum*, was identified as (+)-(5*R*,10*S*)-11,12,14-trihydroxy-8,11,13-abietatriene-3,7-dione. The A ring showed a boat conformation.

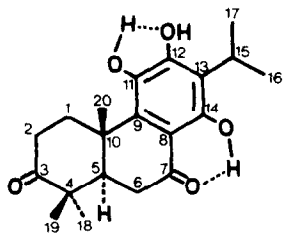
INTRODUCTION

During our research work on the flavonoid composition of some species of *Salvia* [1, 2], we have isolated from a chloroform extract of *S. candelabrum* leaves, a new diterpene, to which structure **1** and the trivial name candelabrone were assigned. Similar compounds (coleons) are known in the Labiatae family. For instance coleon V (11,12,14-trihydroxy-8,11,13-abietatriene-6,7-dione) and its tautomer coleon U (6,11,12,14-tetrahydroxy-5,8,11,13-abietatetraene-7-one), with structures closely related to candelabrone, have been previously isolated from *Plectranthus myrianthus* [3].

The present work describes the assignment of structure **1** to the new diterpene from a study of its spectra (largely NMR).

RESULTS AND DISCUSSION

The empirical formula $C_{20}H_{26}O_5$ was confirmed by elemental analysis (C, H) and mass spectrometry (*m/z* 346, base peak). The UV spectrum obtained in MeOH, and the shifts due to the addition of suitable shifts reagents [4],



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revealed the presence of an aromatic *o*-dihydroxy group, as well as the occurrence of an *o*-hydroxyaryl ketone (Table 1). The IR spectrum confirmed the presence of hydroxyl (3250 cm^{-1} *br d*) and carbonyl (1690 and 1600 cm^{-1}) functions. The 80 MHz ^1H NMR spectrum ($CDCl_3$) showed the presence of an isopropyl group (doublet at $\sim\delta$1.3, multiplet at 3.3), three additional singlet methyls (two at $\sim$1.1 and another at $\sim$1.5), and three hydroxyl protons (at 5.0, 5.9 and 13.4). The very deshielded position of the last hydroxyl proton, together with its slow exchange with D_2O, confirmed the existence of a 2-hydroxyphenylketone moiety. The 100 MHz ^{13}C NMR spectrum exhibited the expected 20 peaks (two of them were very close and could not be resolved at lower field), including two carbonyl carbons (at δ219.5 and 205.2), six non protonated aromatic carbons and three methylene carbons at 35.9, 37.2 and 37.8. Carbon multiplicities were determined using the SEFT (Spin echo Fourier transform) technique [5]. All this evidence pointed to a coleon structure. Although a C-3 keto group has not been previously reported in coleons, it is biosynthetically sound in diterpenoids, and could easily explain the very deshielded carbonyl carbon at δ219.5 [6].

Confirmation of the proposed structure **1** came from analysis of the 250 MHz 2D COSY spectrum (Fig. 1) and the 400 MHz ^1H NMR spectrum (Fig. 2). The proton connectivities revealed by the COSY spectrum allowed the assignements shown in Table 2 (spectral parameters refined by iterative simulation).

The starting point of the spectral assignement was the presence of two independent spin subsystems, one consisting of three protons (H-5, H-6α and H-6β) and the other having four protons (H-1α, H-1β, H-2α and H-2β), with no

Table 1. UV spectra of compound **1** [λ_{max} nm (log ε)]

MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$ + HCl	+NaAcO	+NaAcO + H_3BO_3
289 (3.79)	251	254	293	256	250
352 (3.6)	340	295	314	333	340
	355sh	340	342	368	392
		400	414		

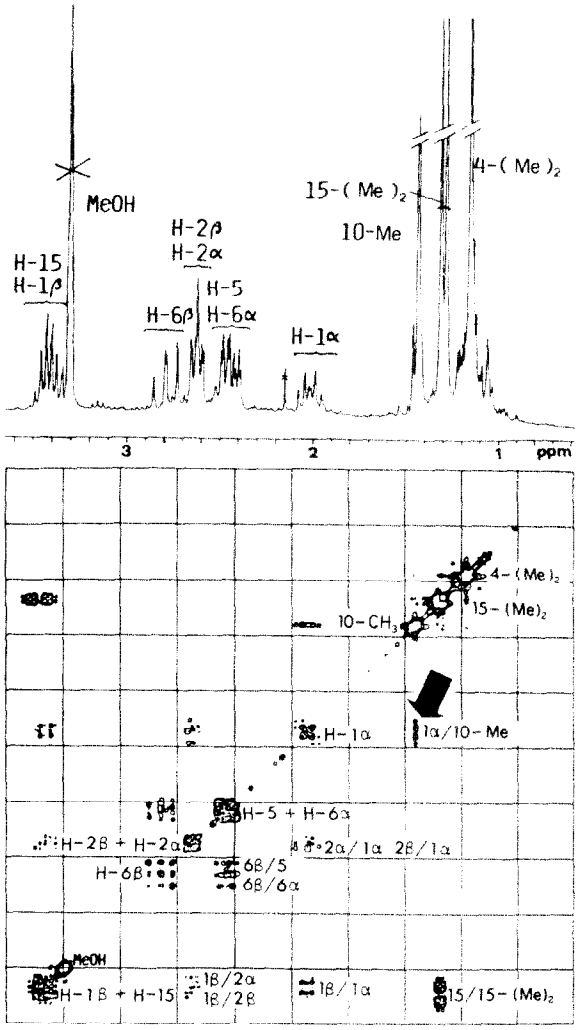

Fig. 1. 250 MHz 2D COSY ^{1}H NMR spectrum of **1** in CD_3OD, obtained using the long range COSYLR pulse sequence [11]. Proton connectivities shown: upper left, three-spin subsystem H-5, H-6α, H-6β; lower right, four-spin subsystem H-1α, H-1β, H-2α, H-2β. Note the long range coupling between H-1α and the angular methyl group (arrowed cross peaks).

interrelating cross peaks in the COSY spectrum. In the three-proton subsystem, a well resolved double doublet at δ2.78 showed two large couplings (J_{gem} = −16.6 Hz, J_{vic} = 14.6 Hz). This clearly established a *trans*-diaxial relationship between the δ2.78 proton and its vicinal partner, and therefore the A/B ring junction was recognised as *trans*, with the individual assignments shown in Table 2. No such large coupling was present in the four-proton subsystem, which therefore lacks *trans*-diaxial pairs of protons. This is not possible for a six membered A ring frozen in the chair conformation. Therefore the A ring is in the boat (or twist) conformation (Fig. 3), as in the case of the related diterpenoid barbatusin [7] and derivatives [8].

The assignment of the individual protons in the four-spin subsystem is based on the presence of an unresolved long range coupling between the angular methyl H-20 (δ1.430) and the double triplet at 2.012. This long range

Table 2. Assigned ^{1}H NMR spectrum of **1** (400.14 MHz, CD_3OD)*

Proton†	δ/ppm‡	m§	J(Hz)‖
H-1α	2.012	*dt*	$J_{1\alpha,1\beta}$ = −13.9
H-1β	3.398	*m*	$J_{1\alpha,2\alpha}$ = 8.3
H-2α¶	2.598	*m*	$J_{1\alpha,2\beta}$ = 8.6
H-2β¶	2.637	*m*	$J_{1\beta,2\alpha}$ = 7.0
H-5	2.421	*m*	$J_{1\beta,2\beta}$ = 5.3
H-6α	2.452	*m*	$J_{2\alpha,2\beta}$ = −14.2
H-6β	2.780	*dd*	$J_{5,6\alpha}$ = 2.9
H-15	3.450	*sept*	$J_{5,6\beta}$ = 14.6
H-16, H-17	1.290	*d*	$J_{6\alpha,6\beta}$ = −16.6
H-18, H-19	1.150	*s*	$J_{15,16} = J_{15,17}$ = 7.1
H-20	1.430	*br s*	$J_{1\alpha,20}$ < 0.1**

*Chemical shifts and coupling constants for protons H-1 to H-15 obtained by iterative simulation analysis (PANIC program) of a resolution enhanced experimental spectrum (see Fig. 1).

†IUPAC numbering.

‡Chemical shifts downfield from TMS.

§Multiplicity explicitly given only for well resolved protons.

‖Couplings rounded to nearest first decimal place.

¶Assignments may be interchanged.

**Unresolved coupling even at a digital resolution of 0.01 Hz, confirmed by a cross peak in the 2D COSY spectrum and by a decoupling experiment, in which irradiation at H-1α resulted in a significant narrowing of the H-20 methyl singlet.

coupling was clearly demonstrated in the 2D COSY spectrum (Fig. 1, arrowed cross peaks) and by a specific decoupling experiment, in which irradiation at the double triplet produced a considerable narrowing of the H-20 singlet. Since these four-bond couplings through sp^3 carbons require coplanarity (*W* pathway) of the bonds connecting the protons involved, the double triplet was assigned to H-1α, which in any conformation is *anti* to the C-10/C-20 σ bond. The geminal proton H-1β was immediately recognised as the signal at δ3.398 (overlapping the isopropyl methine H-15) by the large, negative geminal coupling constant ($J_{1\alpha,1\beta}$ = −13.9 Hz). The rather deshielded position of H-1β can be due to its forced coplanarity with the aromatic C ring and its close proximity to the oxygen lone pairs of the C-11 hydroxyl group, known as 'rabbit ear effect' [9, 10].

The remaining two protons of this four-spin subsystem, namely H-2α and H-2β, appeared very strongly overlapped even at 400 MHz. Nevertheless, iterative simulation allowed spectral analysis, yielding the shifts and couplings shown in Table 2. However, the lack of a fixed *trans*-diaxial relationship between one of them and any of its H-1 neighbours resulted in the four $J_{1,2}$ couplings appearing within the narrow range 5.3–8.6 Hz. Therefore, individual proton assignements within this C-2 methylene are only tentative.

Comparison of the specific rotation of **1**, $[\alpha]_D^{30}$ = +160°, with those of hinokione ($[\alpha]_D$ = +115.6° [12] and 111.9° [13]) and 12-*O*-methyl-spruceanone ($[\alpha]_D$ = 90.5° [14]), having a ketone function in the C-3 position, and with those of demethylcryptojaponol ($[\alpha]_D^{26}$ = +31.2° [15]) and cleistantha-8,11,13-trien-7-one ($[\alpha]_D^{25}$ = +35.5 [16]), having a ketone function in the C-7 position, indicates the absolute configuration shown in **1** (5*R*, 10*S*). Moreover, the ORD spectrum of **1** in methanol

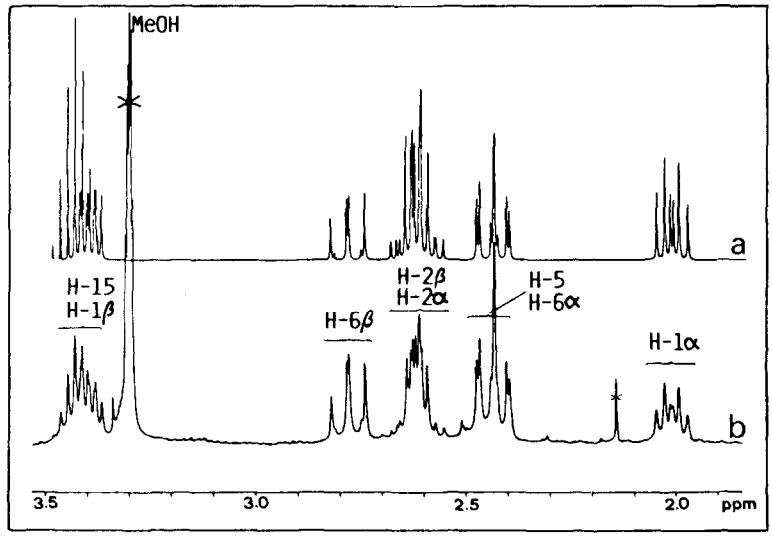

Fig. 2. Resolution enhanced 400 MHz ^{1}H NMR spectrum of **1** in CD_3OD (methyl protons not shown). (a) Experimental spectrum. (b) Final iteration of assigned simulated spectrum (refined chemical shifts and coupling constants given in Table 2). The iterative simulation was carried out using program PANIC.

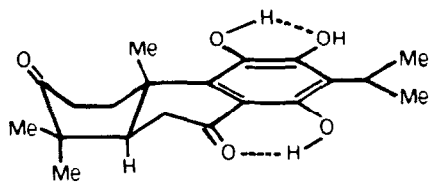

Fig. 3. The proposed conformation of **1**.

given in the Experimental, clearly displays a long wave length positive Cotton effect ($[\alpha]_{max}$ at 390 nm $[\alpha]_0$ at 377 nm and $[\alpha]_{min}$ at 325 nm), undoubtedly associated with the conjugated chromophore, i.e. the aryl ketone at C-7. The related diterpenoid, cleistantha-8,11,13-trien-3,7-dione, known to belong to the 'regular' diterpenoid absolute configuration, has also been reported [17] to show a positive Cotton effect at 338 nm ($[\theta]_{338}$ = +9924). Therefore the absolute configuration of candelabrone is 5*R* 10*S*, as shown in **1** and Fig. 3.

EXPERIMENTAL

Plant material. S. candelabrum was collected near Albuñol (Granada-Spain), authenticated by Prof. M. Ladero (Faculty of Pharmacy, Salamanca, Spain) and a voucher specimen was deposited in the BCF Herbarium (Botany Dept., Fac. Pharmacy, Univ. Barcelona) with the no. 32591.

Isolation. The air-dried powdered leaves (*ca* 200 g) were successively extracted with petrol, $CHCl_3$, MeOH and MeOH–H_2O (1:1). The MeOH soluble fraction of $CHCl_3$ extract yielded 140 mg of **1** by means of CC on polyamide CC-6, eluting with hexane–$CHCl_3$ (99:1) and increasing the solvent polarity by hexane decrease and addition of MeCOEt, MeOH and Me_2CO; silica gel eluting with $CHCl_3$–MeOH (99:1) and EtOAc–petrol (7:3); and Sephadex LH-20, eluting with MeOH.

Physical and spectral data. Mp 224–226° uncorr. (MeOH). UV, see Table 1. IR, ν_{max}^{KBr} cm^{-1}: 3600–3000 (OH's), 1690 (>C=O at C-3), 1600 (chelated >C=O at C-7). EIMS (direct insert probe) 70 eV, *m/z* (rel. int.): 346 $[M]^+$ (100), 331 $[M-Me]^+$ (76), 303 $[M-iPr]^+$ (10), 289 (6), 275 (4), 248 (5), 233 (8), 219 (6), 165 (4), 135 (3), 115 (5). ^{1}H NMR (80 MHz, $CDCl_3$): δ1.13 (6H, *s*, H-18 and H-19), 1.35 (6H, *d*, *J* = 7 Hz, H-16 and H-17), 1.40 (3H, *s*, H-20), 1.9–2.4 (1H, *m*, H-1α), 2.5–2.9 (5H, *m*, H-2α, H-2β, H-5, H-6α and H-6β), 3.0–3.5 (2H, *m*, H-1β and H-15), 5.05 (1H, *s*, C_{12}-OH), 5.95 (1H, *s*, –OH at C-11), 13.40 (1H, *s*, –OH at C-14). ^{1}H NMR (400 MHz, CD_3OD): see Fig. 2 and Table 2. ^{13}C NMR (100 MHz, CD_3OD): δ18.93 (*q*, C-20), 21.01 (*q*, C-16), 21.03 (*q*, C-17), 21.77 (*q*, C-19), 26.28 (*d*, C-15), 27.79 (*q*, C-18), 35.94 (*t*, C-2), 37.23 (*t*, C-6), 37.79 (*t*, C-1), 40.67 (*s*, C-10), 48.61 (*s*, C-4), 50.90 (*d*, C-5), 109.69 (*s*, C-8), 120.94 (*s*, C-13), 136.66 (*s*, C-11), 139.14 (*s*, C-9), 156.80 (*s*, C-14), 160.96 (*s*, C-12), 205.17 (*s*, C-7), 219.46 (*s*, C-3). ORD (MeOH, *c*0.0436): $[\alpha]_{30}$ (λ nm) = +160° (589), +984° (390), 0° (377), −6979° (325). (Found: C, 68.98; H, 7.50. $C_{20}H_{26}O_5$ requires: C, 69.32; H, 7.55%).

REFERENCES

1. Camarasa, J., Cañigueral, S., Iglesias, J. and Marín, E. (1982) *Plantes Méd. Phytothér.* **16**, 192.
2. Adzet, T., Cañigueral, S., Iglesias, J. and Manso, D. (1986) *Planta Med.* **52**, 525.
3. Miyase, T., Rüedi, P. and Eugster, C. H. (1977) *Helv. Chim. Acta* **60**, 2770.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
5. Benn, R. and Günther, H. (1983) *Angew. Chem. Int. Ed. Engl.* **22**, 350.
6. Pinto, A. C., Peixoto, E. M. and Fiorani, N. G. M. (1984) *Phytochemistry* **23**, 1293.
7. Zelnik, R., Lavie, D., Levy, E. C., Wang, A. H. J. and Paul, I. C. (1977) *Tetrahedron* **33**, 1457.
8. Zelnik, R., Gottlieb, H. E. and Lavie, D. (1979) *Tetrahedron* **35**, 2693.
9. Yoshizaki, F., Rüedi, P. and Eugster, C. H. (1979) *Helv. Chim. Acta* **62**, 2754.
10. Miyase, T., Rüedi, P. and Eugster, C. H. (1977) *Helv. Chim. Acta* **60**, 2789.

11. Bax, A. and Freeman, R. (1981). *J. Magn. Res.* **44**, 542.
12. Matsumoto, T., Usui, S., Kawashima, H. and Mitsuki, M. (1981). *Bull. Chem. Soc. Jpn* **54**, 581.
13. Chow, Y.-L. and Erdtman, H. (1962). *Acta Chem. Scand.* **16**, 1296.
14. Gunasekera, S. P., Cordell, G. A. and Farnsworth, N. R. (1979). *J. Nat. Prod.* **42**, 658.
15. Hueso-Rodriguez, J. A., Jimeno, M. L., Rodriguez, B., Savona, G. and Bruno, M. (1983). *Phytochemistry* **22**, 2005.
16. Pinto, A. C., Patitucci, M. L., Da Silva, R. S., Queiroz, P. P. S. and Kelecom, A. (1983). *Tetrahedron* **39**, 3351.
17. Pinto, A. C., Patitucci, M. L., Zocher, D. H. T. and Kelecom, A. (1985). *Phytochemistry* **24**, 2345.