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Artarborol, a *nor*-Caryophyllane Sesquiterpene Alcohol from *Artemisia arborescens.* Stereostructure Assignment through Concurrence of NMR Data and Computational Analysis

Caterina Fattorusso,[§] Emiliano Stendardo,[§] Giovanni Appendino,[†] Ernesto Fattorusso,*[,§] Paolo Luciano,[§] Adriana Romano,[§] and Orazio Taglialatela-Scafati[§]

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano 49, I-80131 Napoli, Italy, and DISCAFF, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy

fattoru@unina.it

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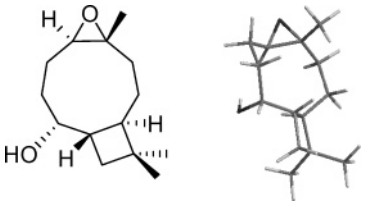
ABSTRACT



A *nor*-caryophyllane derivative, artarborol, has been isolated from wormwood (*Artemisia arborescens*) and its stereostructure established by using a combination of chemical derivatization, NMR data, molecular modeling, and quantum-mechanical calculations. In particular, comparison of experimental ^{13}C NMR data with a Boltzmann-weighed average of ^{13}C NMR chemical shifts, calculated by ab initio DFT method, supported the stereochemical assignment.

Tree wormwood (*Artemisia arborescens* L., Asteraceae, Anthemidae) is an aromatic evergreen shrub endemic to the Mediterranean area. *A. arborescens* is a prolific producer of bitter sesquiterpene lactones but interest in this plant has so far mainly focused on its essential oil, a major source of chamazulene and a potent antiherpetic agent.[1] In the frame of a research project aimed at investigating the molecular basis for the taste properties of sesquiterpene lactones, we have recently undertaken the chemical investigation of a Sardinian chemotype of *A. arborescens* that was identified as a good source of the bitter guaianolides arborescin (**1**)[2] and matricin (**2**).[3]

The high concentration (ca. 0.5% each) and crystal state of these compounds made it possible to develop an expedited isolation procedure that also afforded a *nor*-sesquiterpene alcohol as a major nonlactonic constituent. We have named this compound artarborol (**3**) and detail here the elucidation of its unusual constitution and stereostructure, based on the concurrence of NMR data and ab initio quantum-mechanical calculations. Although apparently a deceptively simple problem, the configurational assignment of artarborol highlights some important pitfalls in the translation of NMR data in configurational terms, and our approach to solve them might have general relevance.

[§] Università di Napoli.
[†] Università del Piemonte Orientale.

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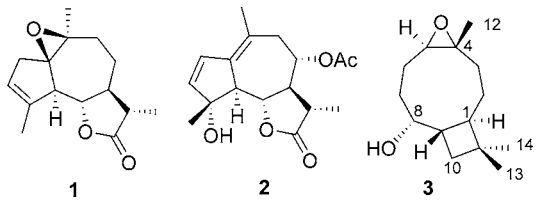
The aerial parts of *A. arborescens* were extracted with acetone at room temperature to afford a black gum, which was next partitioned between water and EtOAc. The organic phase was chromatographed over a silica column using a gradient solvent of increasing polarity. A fraction eluting with hexane/EtOAc 7:3 was further purified by normal-phase HPLC (eluent: hexane/EtOAc 65:35) to give artarborol (**3**, 0.05%) as a colorless amorphous solid.



The molecular formula of artarborol (**3**), $C_{14}H_{24}O_2$, deduced on the basis of HR-EIMS, implied the presence of three unsaturation degrees. A preliminary inspection of the ^{13}C NMR spectrum of **3** (C_6D_6, Table 1), whose highest signal resonated at δ_C 71.4, excluded the presence of double and triple bonds and, consequently, suggested a tricyclic structure for artarborol. The ^1H NMR spectrum of artarborol, recorded in C_6D_6 at 700 MHz (Table 1), showed a number of well resolved multiplets between δ_H 0.96 and 3.40 and three methyl singlets at δ_H 0.86, 0.91, and 1.10. Remarkably, accurate inspection of a 2D NMR COSY spectrum revealed that all the proton multiplets belonged to a single spin system (Figure 1). After association of all the proton signals with those of the directly linked carbons by analysis of the 2D HSQC spectrum, we turned our attention to the 2D HMBC experiment (Figure 1).

Table 1. ^1H NMR and ^{13}C NMR Data for Artarborol (**3**) Recorded in C_6D_6 at 700 MHz for ^1H and 175 MHz for ^{13}C

carbon no.	δH (mult., *J* in Hz)	δC (mult.)
1	1.81 (t, 9.5)	43.9 (CH)
2a	1.37 (td, 14.5, 3.5, 3.5)	28.1 (CH_2)
2b	1.12 (overlapped)	
3a	1.94 (td, 13.0, 3.5, 3.5)	40.3 (CH_2)
3b	0.96 (dt, 13.0, 13.0, 3.5)	
4		58.3 (C)
5	3.10 (dd, 9.0, 5.0)	61.2 (CH)
6a	2.16 (m)	25.1 (CH_2)
6b	1.22 (overlapped)	
7a	1.62 (m)	30.8 (CH_2)
7b	1.20 (overlapped)	
8	3.36 (dd, 9.5, 1.5)	71.4 (CH)
9	1.68 (dq, 9.5, 1.5)	47.4 (CH)
10	1.47 (d, 9.5)	36.9 (CH_2)
11		32.9 (C)
12	1.10 (s)	16.5 (CH_3)
13	0.86 (s)	22.5 (CH_3)
14	0.91 (s)	29.7 (CH_3)

Inspection of the $^{2,3}J_{H\rightarrow C}$ peaks exhibited by the three methyl protons proved to be sufficient to deduce the planar

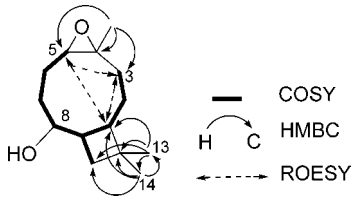


Figure 1. COSY and key HMBC and ROESY correlations detected for artarborol (**3**).

structure of artarborol (**3**). In particular, the methyl protons resonating at δ_H 1.10 (C-12) were coupled to C-3 and to two oxygenated carbons (δ_C 61.2, CH; δ_C 58.3, C), whose relatively high-field resonances are typical of the epoxide ring. The CH_3-13 and CH_3-14 methyls were mutually coupled and both coupled to carbons at δ_C 43.9 (CH), 36.9 (CH_2), and 32.9 (C). Integration of these HMBC correlations in the framework of the proton spin system identified by the COSY experiments implied the presence of fused cyclobutane and cyclononane rings. *nor*-Caryophyllane derivatives, as artarborol (**3**), are very rare in nature and, to our knowledge, only four members of this class have been reported so far.[4]

Five out of the fourteen carbons of artarborol are stereogenic, and their location on a medium-size ring makes the configurational issue challenging, also because in natural caryophyllanes both the cis and the trans junction of rings adjacent to the nine-membered core are possible.[5]

The 2D ROESY experiment was instrumental to reduce the number of possible stereoisomers (Figure 1). Indeed, the ROESY cross-peak between the epoxide proton at δ_H 3.10 and the signal at δ_H 0.96 (H-3b) clearly indicated the trans junction of the epoxide ring. Analogously, the trans junction between the carbocyclic rings was deduced by cross-peaks of the two methyls at C-11: the methyl at δ_H 0.86 showed spatial coupling with H-9 (δ_H 1.68), while the methyl resonating at δ_H 0.91 was coupled to the signal at δ_H 1.81 (H-1). A further reduction of the number of stereoisomers was accomplished by application of the modified Mosher's method to assess the absolute configuration at C-8.[6] Two aliquots of artarborol (**3**) were dissolved in dry pyridine and allowed to react overnight with (*R*)- and (*S*)-MTPA chloride, affording the (*S*)- and (*R*)-MTPA esters **3a** and **3b**, respectively. Analysis of the $\Delta\delta$ ($S-R$) values according to the Mosher model (Figure 2) pointed to an *R* configuration for C-8 of **3**.

At this stage, only the four stereoisomers **A**−**D**, shown in Figure 3, remained as possible candidates. In principle, a further selection could be made by analyzing the pattern of scalar coupling constants and/or of additional spatial cou-

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(6) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

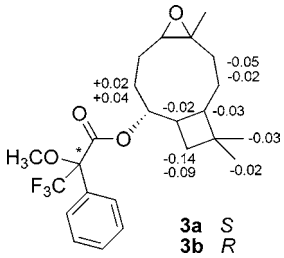

Figure 2. $\Delta\delta$ ($S-R$) values (in ppm) for MTPA ester derivatives of artarborol.

plings on the entire molecule. However, these data could not be translated in terms of configuration without a more detailed knowledge on the conformational features of the stereoisomers **A**−**D**.

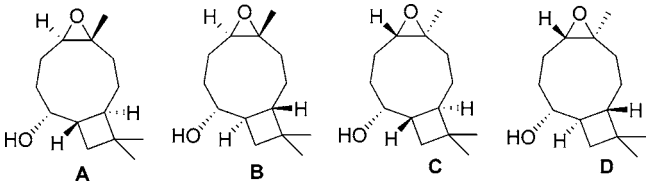

Figure 3. The four candidate structures for artarborol.

To solve this issue, these molecules were subjected to conformational search using the simulated annealing procedure (InsightII Software Package), obtaining a set of conformations optimized using the consistent force field (CFF) and a quasi-Newton−Raphson minimization method until the maximum rms derivative was less than 0.001 kcal/mol. In this way, the resulting conformers could be pooled into families and ranked on the basis of their conformational energy values. To simulate the same environment embedding the molecule during the NMR analysis, a distance-dependent dielectric constant set to the value of benzene (ϵ 2.3) was used during the calculations. The resulting set of conformations was filtered taking advantage of the very low value of $J_{H-8/H-9}$ (1.5 Hz), that pointed to a dihedral angle near 90° between these protons. All the reasonably populated conformations for stereoisomers **B** (3 conformers, Supporting Information) and **D** (2 conformers, Supporting Information) exhibited a dihedral angle around 160°, incompatible with the observed value of the coupling constant and were therefore discarded. The two remaining candidates **A** and **C** differed only for the absolute configuration of epoxide ring carbons and involved stereogenic centers three carbons away from the system C-8/C-9/C-1, whose absolute configuration has been concluded above. On the other hand, the ROESY spectrum of **3** revealed an unexpected and remarkably intense spatial coupling between H-5 and H-1 (Figure 1), resulting from a transannular proximity of these two protons, otherwise separated by five carbon atoms. Analysis of the range of

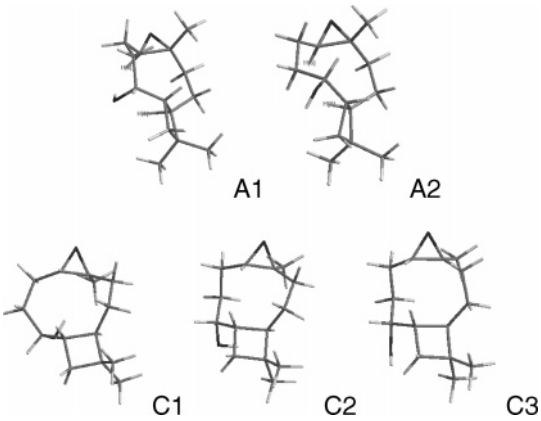

Figure 4. Minimum energy conformations calculated for structures **A** (A1 and A2) and **C** (C1, C2, and C3) (see Figure 3).

conformations available for candidates **A** and **C** (Figure 4) proved that only minimum energy conformers for stereoisomer **A** (A1-A2) can be compatible with the above ROE contact, and, consequently, structure **A** was assigned to artarborol.

To support this stereochemical assignment, the recent methodology based on a quantum-mechanical prediction of ^{13}C NMR chemical shifts was employed,[7] complementing the computational analysis with ab initio calculations of electronic distribution. Since ^{13}C NMR chemical shifts are spread over a large spectral window and are relatively insensitive to solvent shifts, the accurate prediction of ^{13}C NMR chemical shifts is a good test to prove the compatibility of a proposed structure with observed NMR data or to choose among alternative structural hypotheses. As an interesting example, the very recent reassignment of the structure of hexacyclinol, based on prediction of ^{13}C NMR data alone, could be cited.[8] Among the several computational possibilities, the gauge including atomic orbitals (GIAO) calculation of ^{13}C NMR chemical shifts by ab initio density functional theory (DFT) method using 6-31G(d) basis set has been found to give very good results with nonpolar compounds containing only sp^3 carbon atoms.[9] Artarborol (**3**) fits very well with these features and, consequently, the method could be conveniently applied to predict its ^{13}C NMR resonances. However, since the macrocyclic ring system of artarborol is relatively flexible, the ab initio calculations had to be applied to the previously calculated minimum energy conformers: A1 and A2 for **A** (which differ in a flip of the cyclobutane ring and consequent slight rotation of the C-8 to C-10 carbons); C1, C2, and C3 for **C** (also differing in a rotation of the C-8 to C-10 moiety) (Figure 4). Thus, each conformation was analyzed separately, the geometries were fully optimized, and then the NMR chemical shifts were calculated at the same level with the GIAO option using the

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(8) Rychnovsky, S. D. *Org. Lett.* **2006**, *8*, 2895−2898.
(9) Bifulco, G.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron Lett.* **2003**, *44*, 7137−7141.

MPW1PW91/6-31G(d,p) DFT method.[10] Results obtained are collected in Supporting Information (Table S1).

Thermochemical calculations at the same ab initio level in the harmonic approximation of the vibrational modes allowed the evaluation of the standard Gibbs free energy for all the conformers, at the NMR recording temperature of 298 K. Then, a Boltzmann weighted average of ^{13}C NMR chemical shifts for any given carbon atom of low-energy conformers was calculated for each configuration, using the ab initio standard free energies as weighting factors.[11] All these calculations were carried out using the Gaussian 03 program (see Supporting Information). The total CPU time for each molecule was approximately 60 h using a computer incorporating a Pentium-4 processor.

The experimental shifts were plotted against the calculated shifts, and the least-squares fit values of slope, intercept, and correlation factor (r^2) were determined (A: slope = 0.9598, intercept = 1.5986, r^2 = 0.9967. C: slope = 0.9308 intercept = 2.9752, r^2 = 0.9713). To cancel any systematic error present in the calculation, the calculated shifts for each stereoisomer were corrected using the above slope and intercept, obtaining a set of corrected ^{13}C NMR shift. The difference plots reported in Figure 5 were determined by subtracting the corrected chemical shifts from the experimental chemical shifts. For structure A, the average deviation $|\Delta\delta|$ was of 0.77 ppm and only one atom (C-10) showed a difference around 2 ppm; conversely, for structure C the average deviation $|\Delta\delta|$ resulted to be of 2.11 ppm and four atoms (C-3, C-6, C-7, C-12) showed a difference higher than 3 ppm. Therefore, the results of ab initio calculation fully supported the previous assignment of structure A to artarborol.

In conclusion, on the basis of a series of NMR data and computational calculations, we have assigned the absolute configuration to the five chiral carbons of artarborol (**3**), validating this approach for medium-seized compounds, a "difficult" class of compounds for configurational assignments. The full consistency between the spectroscopic analysis and the quantum-mechanical calculations validates methodology based on ab initio prediction of ^{13}C NMR chemical shifts as a screening tool for structural hypotheses. In particular, the approach followed in the present paper seems well suited for relatively flexible medium-sized terpenoid skeleta like germacranes, humulanes and caryophyllanes, where NOE interactions can be difficult to translate into configurational assignments because of the existence of dynamic equilibria. Further applications of this approach will be reported in due course.

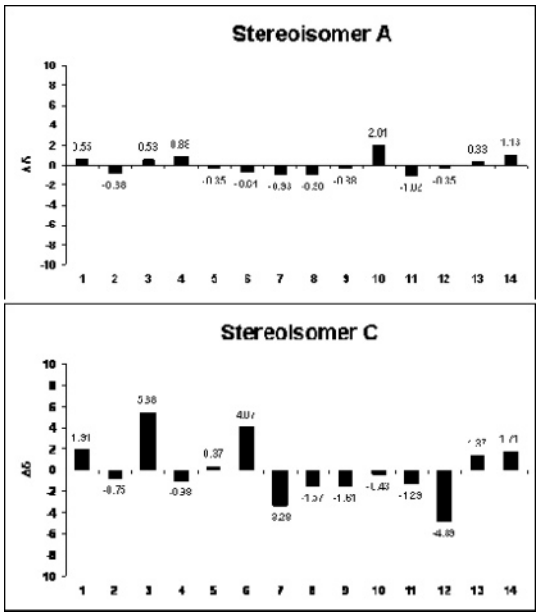

Figure 5. Difference plots determined by subtracting corrected chemical shifts from experimental chemical shifts for **A** and **C**.

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Supporting Information Available: Experimental details, 1D and 2D NMR spectral data for artarborol, minimum energy conformations for stereoisomers **B** and **D**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Barone, G.; Duca, D.; Silvestri, A.; Gomez-Paloma, L.; Riccio, R.; Bifulco, G. *Chem.−Eur. J.* **2002**, *8*, 3240−3245.