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Two New Flavonoids from *Bonannia graeca*: a DFT-NMR Combined Approach in Solving Structures

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Two new cyclized *C*-geranylated flavonoids, the dihydroflavonol bonanniol C (**4a**) and the flavanone bonannione B (**6a**), were isolated as minor compounds from the aerial parts of *Bonannia graeca* (Umbelliferae). Their structures were elucidated by a combined approach of extensive spectroscopic means and quantum mechanical methods.

Introduction

Bonannia graeca (L.) Halácsy, belonging to the Umbelliferae family, is a rare plant growing in several Mediterranean areas as the southern parts of Italy and Greece. It is known for its toxicity against herbivores and for causing the death of lambs feeding on the aerial parts during its blossoming season (June–July). Our previous phytochemical investigations of this species allowed us to isolate a new irregular diterpene, bonandiol[1] and three new *C*-geranylated flavonoids: bonannione A (**1**), bonanniol A (**2**) and bonanniol B (**3**), depicted in Figure 1.[2] These metabolites are particularly interesting for their biological properties. Recently, bonannione A and bonanniol A were shown to have high cytotoxicy,[3] and the latter showed good *anti*-HIV activity.[4] Since we could obtain a larger amount of plant material, we decided to reinvestigate this plant.

Here we report the isolation and characterization of two new minor flavonoids from *B. graeca*. This structure elucidation was quite intriguing, and required a tandem approach between the usual spectroscopic means (1D and 2D NMR) and quantum chemical calculation of the structures and of their properties at the DFT level. Using this approach it is possible to derive the stereostructures of unknown compounds by comparing the experimental NMR spectroscopic data with the corresponding calculated properties for all the possible stereoisomers.[5]

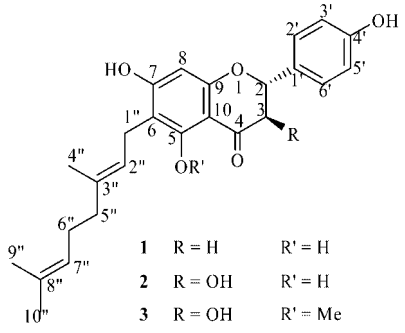

Figure 1. Structures of bonannione A (**1**), bonanniol A (**2**) and bonanniol B (**3**).

Result and Discussion

Serial chromatographic purifications of the acetone extract of *Bonannia graeca* allowed us to isolate two new products from the subfraction eluted with petroleum ether/EtOAc (3:2).

The elemental analysis and ESI-MS of the first one are in agreement with a formula of $C_{25}H_{28}O_7$, and its IR spectrum shows the presence of hydroxy groups (3385 cm^{-1}), a chelated carbonyl group (1642 cm^{-1}), and aromatic rings (1617 cm^{-1}). The ^1H and ^{13}C NMR spectra show signals for a *para*-substituted aromatic ring [δ_H = 7.35 (br. d, 2 H, H-2′ and H-6′) ppm, δ_C = 129.0 (d, C-2′ and C-6′) ppm, δ_H = 6.79 (br. d, 2 H, H-3′ and H-5′) ppm, δ_C = 115.7 (d, C-3′ and C-5′) ppm] bearing an oxygenated function [δ_C = 156.6 (s, C-4′) ppm], for an aromatic methine group [δ_H = 5.99 (s, 1 H, H-8), δ_C = 96.8 (d, C-8) ppm], for other aromatic carbon atoms characteristic of a 5,7-dioxygenated flavonoid, for the oxygenated methine group [δ_C = 83.0 (d, C-2) ppm] at δ_H = 4.95 (d, H-2β) ppm coupling with the proton at δ_H = 4.48 (d, H-3α) ppm on the oxygenated methine group at δ_C = 72.4 (t, C-3) ppm, and for a carbonyl group

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[δ_C = 195.9 (s, C-4) ppm] whose deshielding is owing to the chelation effect of the 5-OH group. Consequently, this product shows an identical flavonic oxygenated pattern of bonanniol A (**2**). In addition, the ^1H and ^{13}C NMR signals of the side chain indicate the presence of a trisubstituted double bond [δ_H = 5.07 (br. t, 1 H, H-7′′) ppm, δ_C = 123.5 (d, C-7′′) ppm, δ_C = 132.3 (s, C-8′′) ppm] bearing two methyl groups [δ_H = 1.59 (s, 3 H, H_3-9′′) ppm, δ_C = 25.6 (q, C-9′′) ppm, δ_H = 1.67 (s, 3 H, H_3-10′′) ppm, δ_C = 17.6 (q, C-10′′) ppm], of another methyl group [δ_H = 1.35 (s, 3 H, H_3-4′′) ppm, δ_C = 19.4 (q, C-4′′) ppm] linked to an oxygenated quaternary carbon atom [δ_C = 80.8 (s, C-3′′) ppm], and of a methylene group [δ_H = 2.85 (dd, 1 H, H-1′′a) ppm, δ_H = 2.68 (dd, 1 H, H-1′′b), δ_C = 24.6 (t, C-1′′) ppm] whose protons are coupling with the proton [δ_H = 3.91 (dd, 1 H, H-2′′) ppm] of the oxygenated methine group at δ_C = 67.1 (d, C-2′′) ppm. The chemical shifts of C-2′′ and C-3′′, as well as the downfield shift of H-2′′ [δ_H = 5.09 (dd, 1 H) ppm] in the tetraacetyl derivative, prepared by treatment of this compound with a mixture of Ac_2O/pyridine for 24 h, clearly shows the presence of a secondary hydroxy group at C-2′′ and consequently a dihydropyrane ring closure.

In order to obtain the configurational assignment of the dihydropyrane ring, and considering the unusual experimental $^3J_{H,H}$ coupling values of H-2′′/H-1′′β and H-2′′/H-1′′α, that are 6.1 Hz and 5.3 Hz, respectively, we have performed a conformational search on both the possible stereoisomers **4a** and **4b** depicted in Figure 2 by molecular dynamics at different temperatures (400, 600, and 800 K).

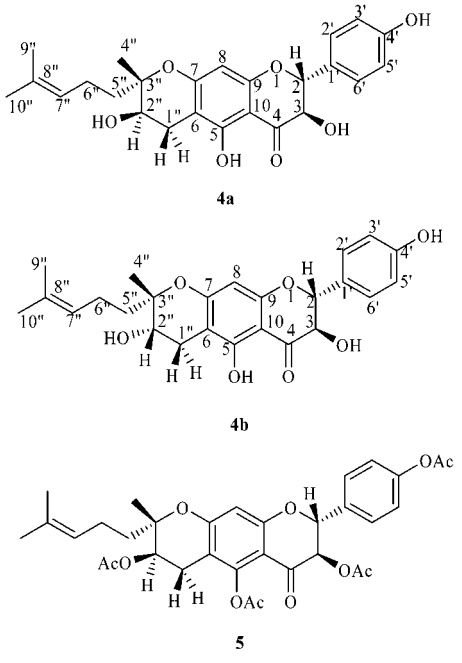

Figure 2. Possible stereoisomers of bonanniol C (**4**) and its acetyl derivative **5**.

For both stereoisomers, two predominant conformers were found (see Figure 3), one of them characterized by the equatorial position of the OH group at C-2′′, and the other by the axial position of the OH group which is balanced by the presence of a hydrogen bond with the oxygen atom at C-3′′. The following quantum mechanical (QM) optimization of the energies and the geometries, performed at the DFT level using the B3LYP functional and the 6-31G(d) basis set, show that structure **4a** is represented by two conformers almost equally represented (ΔH = 0.022 kcal/mol), while for structure **4b** the slightly larger difference in energy of 0.2 kcal/mol accounts for a distribution of 60% and 40% for the conformer with the OH group in the axial position and the one with the OH group in equatorial position, respectively.

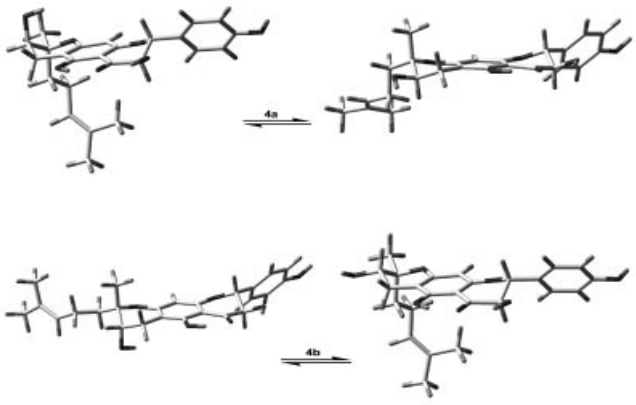

Figure 3. Conformational equilibrium for stereoisomers **4a** and **4b**.

The calculated *J* values, using the B3LYP functional and the 6-31G(d,p) basis set, for the two hypothetical structures **4a** and **4b**, obtained in accordance to their Boltzmann-weighted average, are in both cases in good agreement with the experimental values. In fact, for **4a**, the H-2′′/H-1′′β and H-2′′/H-1′′α calculated couplings are 5.7 Hz and 5.3 Hz, respectively, while for **4b** calculated values of 5.3 Hz for the H-2′′/H-1′′β coupling and 5.0 Hz for the H-2′′/H-1′′α coupling are observed. Even though it is not possible to exclude either of the two relative configurations at this stage, the good reproduction of the medium-intensity experimental *J* values are indicative of the equilibrium described above.

In order to investigate a conformational equilibrium change for this compound, NMR experiments in methanol were performed. The energetic differences for the conformers of **4a** and **4b** were determined at the DFT level simulating the presence of methanol (IEF-PCM), and a new set of coupling constants was calculated taking into account the presence of the solvent and therefore the variations in the Boltzmann distribution of the conformers.

Particularly significant is the experimental value in CD_3OD of the H-2′′/H-1′′β coupling, which increases to 7.27 Hz. Such experimental variation is well reproduced for the hypothetical structure **4a**, with a predicted value of 7.92 Hz. In fact, for **4a**, the conformer having the OH group in the equatorial position is favoured, since the reduced

weight of the hydrogen bond in CD_3OD for the other conformer is not sufficient to balance the presence of the two bulky axial substituents. On the contrary, the experimental value of 7.27 Hz for the H-2′′/H-1′′β coupling is not well reproduced by the calculated data for **4b**, where no significant variation of the conformations, and therefore of the coupling constants, is observed upon change of solvent.

Final evidence for the relative configuration of **4a** is given by a careful examination of the 2D-ROESY NMR experiments. In particular, strong dipolar couplings between H-2′′ and H-5′′ and between H-2′′ and H-1′′α and the lack of a strong ROE effect between H-2′′ and Me-4′′ are in agreement with the H-2′′α arrangement depicted in **4a**.

The absolute configuration of the dihydroflavonol moiety was ascertained because its CD curve, compared with those of bonanniol A (**2**)[2] and literature data[6] is identical. Consequently, this compound was given the C-2(*R*),C-3(*R*),C-2′′(*R*),C-3′′(*S*) stereochemistry (**4a**) or the diastereoisomeric C-2(*R*),C-3(*R*),C-2′′(*S*),C-3′′(*R*) configuration and the trivial name of bonanniol C. To the tetraacetyl derivative was assigned the relative structure **5**.

The elemental analysis and ESI-MS data of the second compound are in agreement with a formula of $C_{25}H_{28}O_6$, and its IR spectrum shows the presence of hydroxy groups (3380 cm^{-1}), a chelated carbonyl group (1658 cm^{-1}), and aromatic rings (1598 cm^{-1}). The ^1H and ^{13}C NMR spectra show a similar flavonic pattern with respect to bonanniol C (**4a**) with the lack of signals due to the β-hydroxy group at C-3. This lack is clearly indicated by the chemical shift of the oxygenated methine group at δ_H = 5.24 (dd, H-2β) ppm [δ_C = 79.0 (d, C-2) ppm] coupling with the geminal protons at δ_H = 2.99 (dd, H-3α) ppm and δ_H = 2.67 (dd, H-3β) ppm [δ_C = 42.7 (t, C-3) ppm]. The ^1H and ^{13}C NMR signals of the side chain indicate the presence of a trisubstituted double bond [δ_H = 5.09 (br. t, 1 H, H-7′′) ppm, δ_C = 123.7 (d, C-7′′) ppm, δ_C = 132.0 (s, C-8′′) ppm] bearing two methyl groups [δ_H = 1.59 (s, 3 H, H_3-9′′) ppm, δ_C = 25.4 (q, C-9′′) ppm, δ_H = 1.65 (s, 3 H, H_3-10′′) ppm, δ_C = 17.5 (q, C-10′′) ppm], of another methyl group [δ_H = 1.28 (s, 3 H, H_3-4′′) ppm, δ_C = 22.4 (q, C-4′′) ppm] linked to an oxygenated quaternary carbon atom [δ_C = 73.8 (s, C-3′′) ppm], and of a methylene group [δ_H = 3.03 (d, 2 H, H-1′′) ppm, δ_C = 25.9 (t, C-1′′) ppm] whose protons are coupling with the proton [δ_H = 4.72 (dd, 1 H, H-2′′) ppm] of the oxygenated methine group at δ_C = 91.3 (d, C-2′′) ppm. The deshielding of the latter carbon signal indicates the presence of a dihydrofurane ring that was confirmed by the correlation in the HMBC spectrum between C-7 and H-2′′. The HSQC and HMBC experiments allowed us to unequivocally assign all the carbon atoms and to confirm that the geranyl chain is linked at C-6 and not at C-8, as clearly indicated by the correlation between C-5 and H-1′′.

Due to the free rotation of the side chain linked at C-2′′ and to the absence of a close stereogenic carbon atom to C-2′′, it was impossible to determine the relative configuration of carbon atoms C-2′′ and C-3′′ with a ROESY experiment. Consequently, we decided to perform a conformational analysis of this compound using a DFT approach in order to have information about the relative configuration of the C-2′′ and C-3′′ carbon atoms.

The QM/NMR relative configurational analysis of this compound was carried out taking into consideration the two possible isomers C-3′′(*S*) (**6a**) and C-3′′(*R*) (**6b**), leaving all the other stereocenters unaltered (Figure 4).

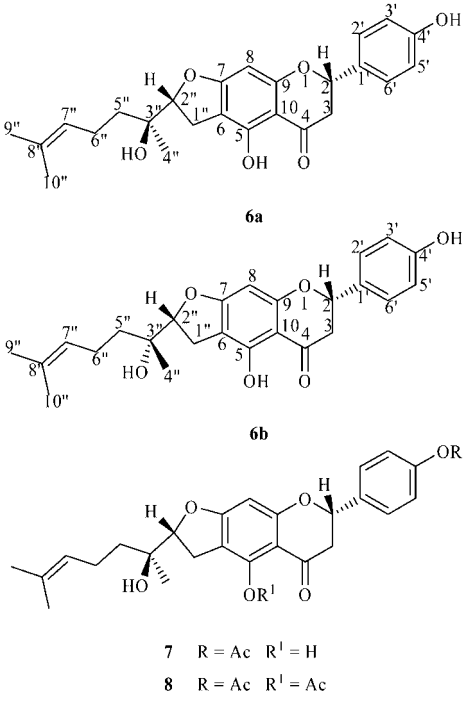

Figure 4. Possible stereoisomers of bonannione B (**6**) and its acetyl derivatives **7** and **8**.

In each case, all the three staggered rotamers, two gauche (g+ and g–) and one *anti* rotamer around the C-2′′/C-3′′ bond were considered (Figure 5).

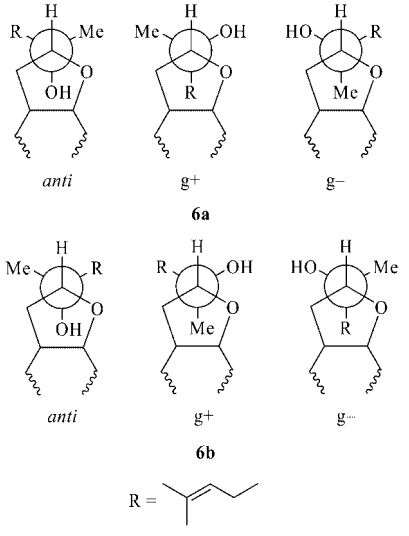

Figure 5. Gauche (g+ and g–) and *anti* rotamers around C-2′′–C-3′′ for compounds **6a** and **6b**. The gauche or *anti* position is with reference to the OH group.

All three conformations of the two stereoisomers **6a** and **6b** were optimized at the DFT level, using the B3LYP functional and the 6-31G(d) basis set. On the resulting geometries we calculated the ^{13}C chemical shifts for each stereoisomer, using the same functional and the 6-31G(d,p) basis, and taking into account the Boltzmann-weighted average derived from the energies of the single conformers.

It is interesting that for isomer **6a**, the *anti* and g+ conformers represent 70% and 28% of the total population, respectively, due to the hydrogen bond between the OH group and the oxygen atom of the ring for both these rotamers. The same consideration may be invoked for the similar trend in the energies and the population distributions observed for **6b**, where the *anti* rotamer accounts for 71% and the g+ rotamer for 28%. This situation is responsible for a large differentiation of the magnetic environment for the methyl group in 4′′-position and the methylene group in 5′′-position in **6a** and in **6b**, as shown in Table 1.

Table 1. Comparison of calculated vs. experimental ^{13}C NMR chemical shifts of stereoisomers **6a** and **6b** in $CDCl_3$.

	^{13}C chemical shifts		
Carbon	**6a**	**6b**	Experimental
2	81.4	81.5	79.0
3	46.2	46.1	42.7
4	187.5	187.5	196.3
5	155.4	155.4	158.3
6	103.2	103.2	105.8
7	161.7	161.7	169.5
8	86.3	86.4	90.5
9	158.4	158.4	163.6
10	101.9	101.9	103.1
1′	127.2	127.2	129.6
2′	122.4	122.5	127.7
3′	107.4	107.4	115.8
4′	150.5	150.5	156.8
5′	111.0	110.9	115.8
6′	123.2	123.3	127.7
1′′	29.1	29.5	25.9
2′′	94.0	94.0	91.3
3′′	73.9	73.9	73.8
4′′	23.1	**19.7**	22.4
5′′	37.3	**40.6**	36.6
6′′	24.8	25.0	21.8
7′′	122.1	122.5	123.7
8′′	128.7	128.0	132.0
9′′	26.5	26.4	25.4
10′′	17.5	17.6	17.5

In fact, while all the other calculated ^{13}C values of **6a** and **6b** differ by a maximum of 0.2 ppm, the C-4′′ signal is predicted at δ = 23.1 ppm for **6a** and at δ = 19.7 ppm for **6b**, while the C-5′′ signal is predicted at δ = 37.3 ppm for **6a** and at δ = 40.6 ppm for **6b** (see Table 1). The subsequent calculation of the difference between the theoretical and experimental values of the ^{13}C chemical shifts ($\Delta\delta$) shows a variation of 0.7 ppm for C-4′′ and C-5′′ in **6a**. Larger $\Delta\delta$ variations, namely 2.7 and 4.0 ppm for C-4′′ and C-5′′, respectively, are found for the hypothetical stereoisomer **6b**.

It is noteworthy that a high accuracy in reproducing the experimental chemical shifts is provided by this level of theory,[5d] which has proved successful in the configurational analysis of several natural compounds.[5] Such accuracy is seldom observed on sp^2 carbon atoms, which are reported in Table 1 together with all the carbon atoms of **6a** and **6b** for completeness, but have not been considered in the configurational assignment in this and in our preceding contributions reported in the literature.[5] Finally, in order to cancel systematic errors due to our methodology, we have also compared the experimental difference between the chemical shifts of C-4′′ and C-5′′ with the corresponding theoretical values for **6a** and **6b**.

Again, the experimental difference of 14.2 ppm is perfectly reproduced for **6a** (14.2 ppm), while the value of 20.9 ppm for **6b** excludes this hypothesis and suggests for the compound the relative configuration of **6a**.

Finally, the absolute configuration of the flavanone moiety was ascertained because its CD curve shows an identical shape compared with those of bonannione A (**1**)[2] and of other C-2(*S*) flavanones[6] (maximum around 330 nm, minimum around 290 nm). Consequently, this compound was given the C-2(*S*),C-2′′(*R*),C-3′′(*S*) stereochemistry (**6a**) or the diastereoisomeric C-2(*S*),C-2′′(*S*),C-3′′(*R*) configuration and the trivial name of bonannione B.

Acetylation of compound **6a** by treatment with a mixture of Ac_2O/pyridine at room temperature for 2 h yielded the C-4′-monoacetyl derivative **7** as clearly indicated by the upfield shift of the C-4′ signal [δ_C = 150.8 (s) ppm] and the downfield shift of the H-3′ and C-5′ signals [δ_H = 7.15 (br. d) ppm] and the C-3′ and C-5′ signals [δ_C = 122.0 (d) ppm]. The same reaction performed for 24 h yielded the 4′,5-diacetyl derivative **8**, as indicated by the upfield shift of the C-4 signal [δ_C = 188.7 (s) ppm] due to the loss of a hydrogen bond. These derivatives allowed us to assign unambiguously the positions of the hydroxy groups in the aromatic rings.

Conclusions

It is our opinion that the biogenetic precursor of both metabolites, bonanniol C (**4a**) and bonannione B (**6a**), is a C-2′′/C-3′′ epoxide cyclizing with the acidic phenolic hydroxy group at C-7 by an S_N2 opening of the epoxide ring. The absolute stereochemistry of the epoxide ring will determine the absolute configuration of carbon atoms C-2′′ and C-3′′ in both compounds; in particular, if the absolute configuration in epoxide precursor is C-2′′(*S*) and C-3′′(*S*), bonannione B will have a C-2′′(*R*),C-3′′(*S*) and bonanniol C a C-2′′(*S*),C-3′′(*R*) absolute configuration, respectively. Of course the cyclization of the epoxide precursor with an opposite stereochemistry C-2′′(*R*),C-3′′(*R*) will give the two diastereoisomers.

It is interesting to note that, according to Baldwin's rules, the favoured 5-*exo*-tet ring-closure reaction should give the dihydrofurane ring. The formation of the dihydropyran by a 6-*endo*-tet cyclization, although disfavoured, takes place, confirming that an enzymatic cyclization does not necessarily follow the principles that govern reactions in solution.[7]

Experimental Section

General: Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were measured with a Jasco P-1010 digital polarimeter. IR spectra (KBr) were obtained with a Shimadzu FTIR 8300 spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution using a Bruker DMX300 instrument at 300 MHz. Heteronuclear 2D ^1H,^{13}C correlations, one-bond HMQC (heteronuclear multiple quantum correlation)[8] and long-range HMBC (heteronuclear multiple bond correlation),[9] were carried out in the ^1H-detected mode with broad-band decoupling in the ^{13}C domain. For ^1H,^{13}C HMBC, the delay for evolution of long-range coupling was set at 65 ms. NMR experiments on bonanniol C (**4a**) and bonannione B (**6a**) were also performed with a Bruker DRX-600 spectrometer at 300 K. All spectra were acquired in the phase-sensitive mode, and the TPPI method was used for quadrature detection in the ω_1 dimension. NMR samples were prepared by dissolving bonanniol C (**4a**) (5.1 mg) in $CDCl_3$ and bonannione B (**6a**) (5.7 mg) in $CDCl_3$ and in CD_3OD (Sigma Aldrich, 99.96% D). The spectra were calibrated using the solvent signal as internal standard (^1H: δ = 7.26 ppm, ^{13}C: δ = 77.0 ppm for $CDCl_3$; ^1H: δ = 3.34 ppm, ^{13}C: δ = 49.0 ppm for methanol). For bonanniol C (**4a**) the ROESY[10] spectra were executed with a number of 16 scans/t_1, a t_{1max} value of 85.31 ms and a mixing time of 600 ms in $CDCl_3$, whereas the ROESY[10] spectra in CD_3OH were executed with a number of 8 scans/t_1, a t_{1max} value of 85.31 ms and a mixing time of 400 ms. For bonannione B (**6a**) the ROESY spectra were executed with a number of 32 scans/t_1, a t_{1max} value of 85.31 ms and a mixing time of 600 ms. The NMR spectroscopic data were processed with a Silicon Graphic Indigo2 Workstation using UXNMR software. ESI-MS was obtained with an Applied Biosystems API-2000 mass spectrometer. Elemental analysis was carried out with a Perkin–Elmer 240 apparatus. CD data were obtained using a Jasco J-715 spectropolarimeter. Merck silica gel (70–2230 mesh), deactivated with 15% H_2O, was used for column chromatography.

Plant Material: The aerial parts of *Bonannia graeca* (L.) Halácsy were collected at Quacella, Piano Battaglia, 80 km SE of Palermo, Sicily, Italy, in July 2006. A typical specimen has been deposited in the Herbarium of the Botanical Science Department, Palermo, Italy (voucher number PAL 06/326).

Extraction and Isolation: The dried and finely powdered aerial parts of *B. graeca* (600 g) were extracted three times with Me_2CO (3 × 5 L) at room temperature for one week. After filtration, the solvent was evaporated, yielding a gum (42 g) which was subjected to chromatography on a dry-packed silica gel column eluting with a solvent gradient from 100% petroleum ether (b.p. 40–60 °C) to 100% EtOAc. The fraction eluted with petroleum ether/EtOAc (4:1) yielded bonandiol (8 g). The fraction eluted with petroleum ether/EtOAc (3:2), was further purified by column chromatography with petroleum ether/EtOAc (7:3) as eluent to afford, in order of increasing polarity, bonannione A (**1**, 2.1 g), bonannione B (**6a**, 80 mg), bonanniol C (**4a**, 50 mg), bonanniol A (**2**, 4.8 g) and bonanniol B (**3**, 1.2 g).

Bonanniol C (4a): Mp. 83–85 °C. $[\alpha]_D^{25}$ = +18.5 (c = 0.54, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 3385, 2923, 2853, 1642, 1617, 1456, 1377, 1260, 1160, 1125 1093 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): see Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz): spectroscopic data and HMBC correlations are reported in Table 3. CD (EtOH): λ_{max} ($\Delta\varepsilon$) = 333 (+5.51), 295 (–13.82) nm. ESI-MS (positive mode): m/z (%) = 479 (27) $[M + K]^+$, 463 (100) $[M + Na]^+$, 441 (14) $[M + H]^+$. ESI-MS (negative mode): m/z (%) = 477 (17) $[M + {}^{37}Cl]^-$, 475 (53) $[M + {}^{35}Cl]^-$, 439 (100) $[M - H]^-$. $C_{25}H_{28}O_7$ (440.49): calcd. C 68.17, H 6.41; found C 68.15, H 6.42.

Acetylation of Bonanniol C (4a): Bonanniol C (**4a**, 20 mg) was dissolved in Ac_2O/pyridine (2:1, 2 mL), and the mixture was maintained at room temperature for 24 h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried with anhydrous Na_2SO_4. Purification by column chromatography (petroleum ether/EtOAc, 4:1) yielded 25 mg of compound **5**.

Compound 5: Amorphous solid. $[\alpha]_D^{25}$ = +77.4 (c = 0.63, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 2922, 2856, 1749, 1698, 1622, 1569, 1469, 1371, 1226, 1195, 1160, 1106 1048 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): see Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz): spectroscopic data and HMBC correlations are reported in Table 3. ESI-MS (positive mode): m/z (%) = 647 (100) $[M + K]^+$, 609 (18) $[M + H]^+$. $C_{33}H_{36}O_{11}$ (608.63): calcd. C 65.12, H 5.96; found C 65.10, H 5.94.

Bonannione B (6a): Mp. 79–81 °C. $[\alpha]_D^{25}$ = –17.7 (c = 3.10, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 3380, 2924, 2853, 1658, 1598, 1517, 1461, 1377,

Table 2. ^1H NMR spectroscopic data of compounds **4**–**8** in $CDCl_3$ (J values [Hz] in parentheses).

H	4a	5	6a	7	8
2β	4.95 d (12.0)	5.37 d (12.0)	5.24 dd (12.9, 3.0)	5.38 dd (12.9, 3.0)	5.40 dd (12.9, 2.7)
3α	4.48 d (12.0)	5.65 d (12.0)	2.99 dd (17.4, 12.9)	3.02 dd (17.4, 12.9)	2.94 dd (17.4, 12.9)
3β	–	–	2.67 dd (17.4, 3.0)	2.78 dd (17.4, 3.0)	2.69 dd (17.4, 2.7)
8	5.99 s	6.41 s	5.94 s	6.00 s	6.33 s
2′,6′	7.35 br. d (8.4)	7.48 br. d (8.6)	7.23 br. d (8.7)	7.46 br. d (8.7)	7.45 br. d (8.6)
3′,5′	6.79 br. d (8.4)	7.16 br. d (8.6)	6.83 br. d (8.7)	7.15 br. d (8.7)	7.14 br. d (8.6)
1′′a	2.85 dd (17.1, 4.8)	2.85 dd (16.4, 4.8)	3.03 d (8.7)	3.06 d (8.7)	3.05 d (8.7)
1′′b	2.68 dd (17.1, 5.4)	2.67 dd (16.4, 4.8)	3.03 d (8.7)	3.06 d (8.7)	3.05 d (8.7)
2′′	3.91 dd (5.4, 4.8)	5.09 dd (4.8, 4.8)	4.72 dd (8.7, 8.7)	4.75 dd (8.7, 8.7)	4.74 dd (8.7, 8.7)
Me-4′′	1.35 s	1.34 s	1.28 s	1.29 s	1.28 s
5′′ (2 H)	1.62 m	1.61 m	1.50 m	1.53 m	1.52 m
6′′ (2 H)	2.11 m	2.13 m	2.09 m	2.12 m	2.12 m
7′′	5.07 br. t (6.9)	5.03 br. t (6.9)	5.09 br. t (6.3)	5.12 br. t (6.3)	5.12 br. t (6.3)
Me-9′′	1.59 s	1.58 s	1.59 s	1.63 s	1.63 s
Me-10′′	1.67 s	1.66 s	1.65 s	1.69 s	1.69 s
OH	11.52 s		12.13 s	12.14 s	–
Ac-4′		2.32 s		2.32 s	2.30 s
Ac-5		2.40 s			2.39 s
Ac-3		2.01 s			
Ac-2′′		2.08 s			

Table 3. ^{13}C NMR spectroscopic data of compounds **4** and **5** in $CDCl_3$.

C	**4a**		**5**	
	ppm	HMBC	ppm	HMBC
2	83.0 d	H3α,H2′,H6′	80.5 d	H3α,H2′,H6′
3	72.4 d		73.3 d	H2β,Ac(^4J)
4	195.9 s	H2,H3α	184.9 s	H8,H2β,H3α
5	161.3 s	OH	149.8 s	Ac(^4J)
6	100.4 s	OH,H8,H2′′,H1′′a,H1′′b	108.4 s	H8,H2′′
7	162.6 s	H8,H1a′′,H1b′′	160.1 s	H8
8	96.8 d		103.0 d	
9	161.3 s	H8	161.1 s	H8,H2
10	100.4 s	OH,H8	106.8 s	H8
1′	128.0 s	H3′,H5′,H2β,H3α	133.0 s	H3′,H5′,H3α,H2β
2′,6′	129.0 d	H3′,H5′,H2β	128.6 d	H3′,H5′,H2β
3′,5′	115.7 d	H2′,H6′	121.8 d	H2′,H6′
4′	156.6 s	H2′,H6′,H3′,H5	151.3 s	H2′,H6′,H3′,H5,Ac(^4J)
1′′	24.6 t		22.7 t	H2′′
2′′	67.1 d	H1′′a,H1′′b,H5′′,Me4′′	68.3 d	Ac(^4J),H5′′,Me4′′
3′′	80.8 s	H1a′′,H1b′′,Me4′′	79.4 s	H2′′,H6′′,H5′′,Me4′′
4′′	19.4 q	H2′′	20.4 q	H2′′
5′′	37.2 t	H6′′,Me4′′	37.0 t	H2′′,H6′′,Me4′′
6′′	21.7 t	H5′′	21.6 t	H7′′,H5′′
7′′	123.5 d	H6′′,Me10′′,Me9′′	123.0 d	H6′′,Me10′′,Me9′′
8′′	132.3 s	H6′′,Me10′′,Me9′′	132.5 s	H6′′,Me10′′,Me9′′
9′′	25.6 q	H7′′,Me10′′	25.6 q	H7′′,Me10′′
10′′	17.6 q	H7′′,Me9′′	17.6 q	H7′′,Me9′′
Ac-4′			169.2 s	Ac
			21.1 q	
Ac-5			168.8 s	Ac
			20.9 q	
Ac-3			169.1 s	H3,Ac
			20.4 q	
Ac-2′′			170.4 s	H2,Ac
			21.0 q	

Table 4. ^{13}C NMR spectroscopic data of compounds **6**–**8** in $CDCl_3$.

C	**6a**		**7**		**8**	
	ppm	HMBC	ppm	HMBC	ppm	HMBC
2	79.0 d	H3α,H2′,H6′	78.7 d	H3α,H2′,H6′	78.9 d	H3α,H2′,H6′
3	42.7 t		43.2 t		44.9 t	
4	196.3 s	H2β, H3α,H3β	195.5 s	H3α,H3β	188.7 s	H3α,H3β
5	158.3 s	OH,H1′′	158.6 s	OH,H1′′	146.6 s	H1′′,Ac
6	105.8 s	OH,H8,H2′′,H1′′	106.1 s	OH,H8,H1′′	115.7 s	H8,H1′′
7	169.5 s	H8,H2′′,H1′′	168.5 s	H8,H2′′,H1′′	166.2 s	H8,H2′′,H1′′
8	90.5 d		90.5 d		96.4 d	
9	163.6 s	H8	163.3 s	H8	164.4 s	H8
10	103.1 s	OH,H3β,H8	103.2 s	OH,H3β,H8	107.8 s	H8
1′	129.6 s	H3′,H5′,H2β,H3α	136.0 s	H3′,H5′,H2β,H3α	136.0 s	H3′,H5′,H2β,H3α
2′,6′	127.7 d	H3′,H5′,H2β	127.0 d	H3′,H5′,H2β	127.3 d	H3′,H5′,H2β
3′,5′	115.8 d	H2′,H6′	122.0 d	H2′,H6′	121.9 d	H2′,H6′
4′	156.8 s	H2′,H6′,H3′,H5′	150.8 s	H2′,H6′,H3′,H5′	150.8 s	H2′,H6′,H3′,H5,Ac
1′′	25.9 t	H2′′	26.0 t	H2′′	26.5 t	
2′′	91.3 d	H1′′,H5′′,Me4′′	91.4 d	H1′′,H5′′,Me4′′	91.1 d	H1′′,Me4′′
3′′	73.8 s	H1′′,H5′′,Me4′′	73.6 s	H1′′,H5′′,Me4′′	73.4 s	H1′′,H5′′,Me4′′
4′′	22.4 q	H2′′	22.6 q		22.4 q	H2′′
5′′	36.6 t	H2′′,H6′′,Me4′′	36.6 t	H2′′,Me4′′	37.0 t	H2′′,Me4′′
6′′	21.8 t	H7′′,H5′′	21.9 t	H5′′	21.8 t	H5′′
7′′	123.7 d	H6′′,Me10′′,Me9′′,H5′′	123.9 d	Me10′′,Me9′′,H5′′	123.8 d	Me10′′,Me9′′
8′′	132.0 s	H6′′,Me10′′,Me9′′	132.2 s	Me10′′,Me9′′	132.3 s	Me10′′,Me9′′
9′′	25.4 q	H7′′,Me10′′	25.6 q	Me10′′	25.6 q	H7′′,Me10′′
10′′	17.5 q	H7′′,Me9′′	17.6 q	Me9′′	17.6 q	H7′′,Me9′′
Ac-4′			169.3 s		169.2 s	Ac
			21.1 q		21.0 q	
Ac-5					168.8 s	Ac
					20.9 q	

1341, 1142, 1094 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): see Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz) spectroscopic data and HMBC correlations are reported in Table 4. CD (EtOH): λ_{max} ($\Delta\varepsilon$) = 330 (+2.51), 293 (–8.59) nm. ESI-MS (positive mode): *m/z* (%) = 463 (100) $[M + K]^+$, 447 (75) $[M + Na]^+$, 425 (32) $[M + H]^+$. ESI-MS (negative mode): *m/z* (%) = 461 (2) $[M + {}^{37}Cl]^-$, 459 (8) $[M + {}^{35}Cl]^-$, 423 (100) $[M – H]^-$. $C_{25}H_{28}O_6$ (424.49): calcd. C 70.74, H 6.65; found C 70.71, H 6.68.

Acetylation of Bonannione B (6a): Bonannione B (**6a**, 20 mg) was dissolved in Ac_2O/pyridine (2:1, 2 mL), and the mixture was maintained at room temperature for 2 h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried with anhydrous Na_2SO_4. Purification by column chromatography (petroleum ether/EtOAc, 4:1) yielded 19 mg of compound **7**. The same reaction maintained at room temperature for 24 h gave, after the usual workup, 21 mg of compound **8**.

Compound 7: Mp. 128–130 °C. $[\alpha]_D^{25}$ = –54.3 (*c* = 0.38, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 3550, 2920, 2853, 1751, 1657, 1620, 1596, 1457, 1377, 1231, 1140, 1094 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): see Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz): spectroscopic data and HMBC correlations are reported in Table 4. ESI-MS (positive mode): *m/z* (%) = 503 (100) $[M + K]^+$, 489 (71) $[M + Na]^+$, 467 (12) $[M + H]^+$. ESI-MS (negative mode): *m/z* (%) = 503 (7) $[M + {}^{37}Cl]^-$, 501 (21) $[M + {}^{35}Cl]^-$, 465 (100) $[M – H]^-$. $C_{27}H_{30}O_7$ (466.52): calcd. C 69.51, H 6.48; found C 69.47, H 6.50.

Compound 8: Mp. 150–152 °C. $[\alpha]_D^{25}$ = –5.3 (*c* = 1.21, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 2922, 2856, 1749, 1698, 1622, 1569, 1469, 1371, 1226, 1195, 1160, 1106 1048 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): see Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz): spectroscopic data and HMBC correlations are reported in Table 4. ESI-MS (positive mode): *m/z* (%) = 547 (12) $[M + K]^+$, 531 (24) $[M + Na]^+$, 509 (100) $[M + H]^+$. ESI-MS (negative mode): *m/z* (%) = 545 (16) $[M + {}^{37}Cl]^-$, 543 (48) $[M + {}^{35}Cl]^-$, 507 (100) $[M – H]^-$. $C_{29}H_{32}O_8$ (508.56): calcd. C 68.49, H 6.34; found C 68.51, H 6.35.

Computational Details: In order to allow a full exploration of the conformational space on bonanniol C (**4a**), MM/MD calculations at temperatures 400, 600, and 800 K (100 ps each) were performed with a Pentium 4 processor at 2.8 GHz using the MMFF force field and the MonteCarlo Multiple Minimum (MCMM) method of the MacroModel package.[11] All the structures thus obtained (in a number of 100) were minimized using the Polak-Ribier Conjugate Gradient algorithm (PRCG, 1000 steps, maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest energy minimum conformer for bonanniol C (**4a**). The initial geometries of the minimum-energy conformers for bonanniol C (**4a**) were optimized at the hybrid DFT B3LYP level using the 6-31G(d) basis set (Gaussian 03 Software Package).[12] GIAO ^1H, ^{13}C and *J*-coupling calculations were performed using the B3LYP functional and the 6-31G(d,p) basis set, using as input the geometry previously optimized at the B3LYP/6-31G(d) level. For these calculations, the IEF-PCM solvent continuum model, as implemented in Gaussian (methanol solvent), was used.[13] For bonannione B (**6a**), MM/MD calculations were not performed and the hypothesized rotamers were directly optimized at the hybrid DFT B3LYP level using the 6-31G(d) basis set (Gaussian 03 Software Package).[12] GIAO ^{13}C calculations were performed using the B3LYP functional and the 6-31G(d,p) basis set, using as input the geometry previously optimized at the B3LYP/6-31G(d) level.

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