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Non-empirical assignment of the absolute configuration of (−)-naringenin, by coupling the exciton analysis of the circular dichroism spectrum and the *ab initio* calculation of the optical rotatory power†‡

Egidio Giorgio,[a] Nunziatina Parrinello,[b] Salvatore Caccamese*[b] and Carlo Rosini*[a]

[a] *Dipartimento di Chimica, Università della Basilicata, via N. Sauro 85, 85100, Potenza, ITALY*

[b] *Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 8, 95125, Catania, ITALY. E-mail: rosini@unibas.it; Fax: +39 0971 202223; Tel: +39 0971 202241*

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The non-empirical assignment of the absolute configuration of (−)-naringenin, the aglycone of (−)-naringin, a flavanone glycoside abundant in the albedo of immature grapefruits and showing several interesting biological properties, has been approached by two different methods: (a) the exciton analysis of the circular dichroism (CD) spectrum and (b) the *ab initio* calculation of the optical rotatory power. Both the methods indicate the configurational correlation (−)/(*S*), as empirically suggested by Gaffield. A comparison of advantages and limitations of the two methods of analysis is also presented.

Introduction

Naringenin is a flavanone formed in the intestinal epithelia by the enzymatic hydrolysis of naringin, its 7-*O*-neohesperidoside (Chart 1). Naringenin possesses chemopreventive potential towards mutagenesis of heterocyclic amines mediated by an isoform of cytochrome P450,[1] inhibits proliferation of a human breast carcinoma cell line, especially when paired with quercetin,[2] and selectively inhibits arachinodate 5-lipoxygenase compared to cyclooxygenase in stimulated rat peritoneal leukocytes.[3] However, in these studies the relevance of the stereochemistry at the C-2 stereogenic centre has not been considered although it is well known that interactions of an enzymatic system leading to the functionalization of a substrate is stereospecific.[4] Thus, the compound has a significant pharmacological interest and in this respect we isolated the diastereomers at C-2 of its glycoside naringin from the albedo of grapefruits.[5] During this work we noticed that (−)-naringenin was previously isolated by enzymatic hydrolysis of (−)-naringin abundant in the immature grapefruits. However, this was not separated from other flavanone glycosides present in the grapefruit. The chiroptical properties of (−)-naringenin obtained in this way were reported but they can be affected by the isolation procedure: *i.e.* unfractionated naringin samples given by hydrolysis contain not only naringenin but also other flavanones, clearly modifying its chiroptical properties.[6] The absolute configuration was however empirically assigned by an extension of the Snatzke rule and the study of the Cotton effects in the circular dichroism spectrum.[7]

The aim of this investigation is to obtain a pure sample of (−)-naringenin and to arrive at a non-empirical assignment of the molecular absolute configuration (AC) of (−)-naringenin **1**, from the analysis of its chiroptical properties. In particular we shall study the Cotton effects, present in its circular dichroism (CD) spectrum and allied to electrically allowed transitions, by means of the coupled-oscillator model due to DeVoe.[8,9] In addition we shall try to confirm the assignment reached above, by means of an approach very recently proposed :[10,11] the *ab initio* calculation of the $[a]_D$. In this way it will be possible not only to safely establish the AC of this interesting natural compound but it will be also possible to make a comparison of advantages and disadvantages of these two different methods of configurational assignment.

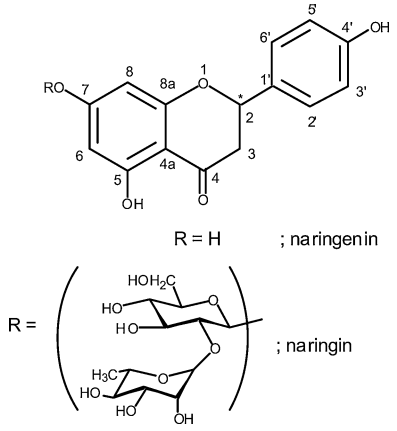

Chart 1

Results and discussion

Isolation of the pure enantiomers of naringenin

An efficient separation of the enantiomers of naringenin was accomplished by enantioselective HPLC on a polysaccharide-derived chiral stationary phase (Chiralcel OD-H) using as a mobile phase *n*-hexane–2-propanol 80 : 20 at a flow rate of 1.3 mL min^{-1}. 2-Propanol was doped with 0.1% of trifluoroacetic acid. In these conditions two major peaks eluted at 8.6 and 10.1 min. A separation factor α of 1.26 and a resolution factor R_s of 1.3 were obtained.[12] Using these experimental parameters we performed a semipreparative chromatography. Through repeated 50 μL injections (0.2–0.3 mg of a solution of commercial naringenin in 2-propanol) and collection of the major chromatographic peaks we isolated 5 mg of (*R*)- and (*S*)-naringenin as the first and the second peak respectively. Details of the optimization of the HPLC procedure for this compound as well as for other flavanones with respect to the use of various chiral stationary phases will be published elsewhere.[13] Thus, their

† This paper is dedicated to Professor Giovanni Gottarelli, an outstanding scientist and a dear friend, on the occasion of his 65th birthday.

‡ Electronic supplementary information (ESI) available: Calculated (CNDO/S-CI) optical data for 2,4,6-trihydroxyacetophenone. See http://www.rsc.org/suppdata/ob/b4/b411110a/

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quantitative chiroptical properties were measured, without any interference due to the presence of other CD-active flavanones.

Circular dichroism spectrum of (−)-naringenin, (−)-1

The absorption and circular dichroism (CD) spectra of (−)-**1**, measured in ethanol between 350 and 210 nm, are collected in Fig. 1.

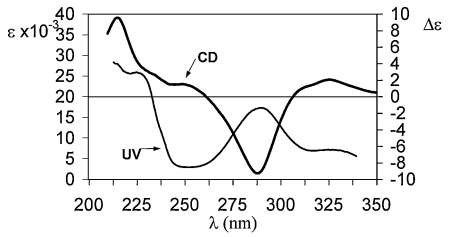

Fig. 1 Absorption (UV, thin line) and circular dichroism (CD, thick line) spectra of (−)-**1** in ethanol.

The UV spectrum shows three main regions of absorption: a first band is present at 330 nm ($\varepsilon = ca.$ 7000), followed by a more intense band at *ca*. 290 nm ($\varepsilon = ca.$ 17 000) while at about 225 nm a third, high-intensity absorption can be observed with ε *ca*. 25 000. In the CD spectrum at least four different Cotton effects can be noticed: the lowest energy one is at about 330 nm ($\Delta\varepsilon$ +2), followed by a strong negative ($\Delta\varepsilon$ –9.5) CD band at 290 nm and by two positive Cotton effects at *ca*. 250 nm ($\Delta\varepsilon$ *ca*. +2, sh) and 215 nm ($\Delta\varepsilon$ +10).

In order to arrive at the configurational determination, the absorption and CD spectra must be carefully analysed and interpreted. The first step of such analysis is the assignment of the observed electronic transitions and Cotton effects. The molecule under investigation, (−)-**1**, can be considered as an aggregate of two different groups, which are electronically separated (*i.e*., these two groups cannot exchange electrons): a 2,4,6-trihydroxyacetophenone chromophore and 4-methylphenol chromophore. The observed optical and chiroptical properties of (−)-**1** derive from the coupling of these two chromophores, which possess electrically allowed transitions: for instance the band at 290 nm possesses ε *ca*. 17 000, therefore the origin of the corresponding Cotton effect could be found in the exciton coupling[8] of its transition dipole with other suitably placed electric transition dipole(s) of the phenyl chromophore. It is nowadays well accepted that the exciton (coupled oscillator) method constitutes a relatively simple, reliable and powerful tool to assign the absolute configuration (AC) of organic molecules. In particular, by means of the DeVoe coupled oscillator (or polarizability) model[9] it is possible to calculate $\Delta\varepsilon$ as a frequency function, *i.e*. the CD spectrum in the range of the electrically allowed transitions can be quantitatively predicted, assuming arbitrarily the molecular AC: thus, from the comparison between predicted and experimental CD a safe AC assignment can be achieved. A correct application of the coupled oscillator methods requires[8,9] the knowledge of the characteristics of the electrically allowed transitions involved (*i.e*. polarization direction, location, allied dipole strength) and of the molecular conformation (structure of the single conformer, conformer distribution). Thus, our analysis of the CD spectrum of (−)-**1** starts with the characterization of the electronic transitions. A first important information can be immediately drawn. In fact, since the UV spectrum of phenol shows [14] strong absorptions only below 240 nm and only a weak band (ε *ca*. 1000) centred at about 270–280 nm, clearly the bands at 330 nm and 285 nm in the absorption spectrum of (−)-**1** are localised on the trisubstituted acetophenone chromophore, whilst below 240 nm, the absorptions are due to the both chromophores. This means that an assignment of the two low-energy transitions can be arrived at by a simple CNDO/S-CI calculation upon the trisubstituted acetophenone chromophore.

MO analysis of the electronic transitions of 2,4,6-trihydroxyacetophenone chromophore

CNDO/S-CI calculations[15] on 2,4,6-trihydroxyacetophenone using default parameters and 60 monoexcited configurations in the CI procedure were carried out using a molecular geometry obtained by MM2 force field[16] calculations: the molecule is planar with hydrogen-bonding between the carbonyl group and a hydroxyl group in the 2 position of the benzene ring: the molecular point group is C_s. In the 380–240 nm spectral range the following electronic transitions have been predicted:

1. An electrically forbidden (A′ symmetry in the C_s group) transition is calculated to occur at 375 nm.
2. A weakly allowed ($f = 0.03$) transition ar 337 nm, polarized in the plane of the aromatic ring, in practice normally (*x*-axis) to the O=C direction (*y*-axis), which reasonably corresponds to the experimental band observed at 330 nm. This transition is due to the MO32–MO33 (HOMO–LUMO) excitation and it is mainly localized on the benzene ring.
3. An allowed ($f = 0.32$) transition at 269 nm, polarized along the O=C (*y*) axis, which reasonably corresponds to the intense band observed at 283 nm in the experimental spectrum. This transition is due to the MO31–MO33 excitation and it involves mainly π orbitals centred on the O=C group and on the C1 and C4 carbon atoms of the benzene ring.
4. A couple of strongly ($f = ca.$ 0.74–0.96) allowed transitions are found in the 225–230 nm region. These two excitations are orthogonally polarized in the plane of the aromatic ring.
5. At shorter wavelengths many other excitations are predicted having in-plane and out-of-plane polarization.

On the basis of these results, as anticipated above, the experimental band at 330 nm is assigned to the HOMO–LUMO transition polarized along the *x*-axis (using our previous definition) whilst the 290 nm band is assigned to the fully allowed OM31–OM33 excitation, polarized along the *y*-axis. Therefore, in order to carry out the AC assignment of (−)-**1**, we could attempt an exciton interpretation of the CD spectrum, by calculating $\Delta\varepsilon(\lambda)$ by means of the DeVoe model, assuming, for instance, (*S*) AC for naringenin and comparing the predicted CD spectrum with that of (−)-**1**. The theoretical CD spectrum comes from the exciton interaction of the 2,4,6-trihydroxyacetophenone chromophore with the 4-methylphenol chromophore, the two chomophores adopting the same relative spatial orientation that they assume in (*S*)-naringenin. So, at least in principle, the entire CD spectrum can be predicted, if all the electronic transitions of the two chromophores are correctly taken in to account. However, from a practical point of view, it is better to limit strongly the spectral range of the calculations, for two main reasons. First of all, the lowest energy transition is only weakly allowed therefore the coupling effect is small leading to a result which can be ambiguous: it has been pointed out that exciton calculations are reliable only when strongly allowed transitions are taken into account.[9d] Second, below 240 nm, several electronic transitions with different polarization have been calculated for 2,4,6-trihydroxyacetophenone chromophore and certainly even the 4-methylphenol one will possess many of them, so the control of the coupling of all these dipoles will be hard to carry out; in other words the prediction of the CD spectrum below 240 nm could be unreliable. Thus it is better to limit our calculations to the 320–250 nm spectral range, *i.e*. attempting at reproducing only the Cotton effect allied to this isolated and fully characterised absorption. This decision will simplify our calculations, without reducing or even affecting the reliability of the configurational assignment.

Geometrical input parameters

Molecular mechanics (MM2 force field)[16] calculations revealed that (*S*)-naringenin exists predominantly as a single conformer (Fig. 2).

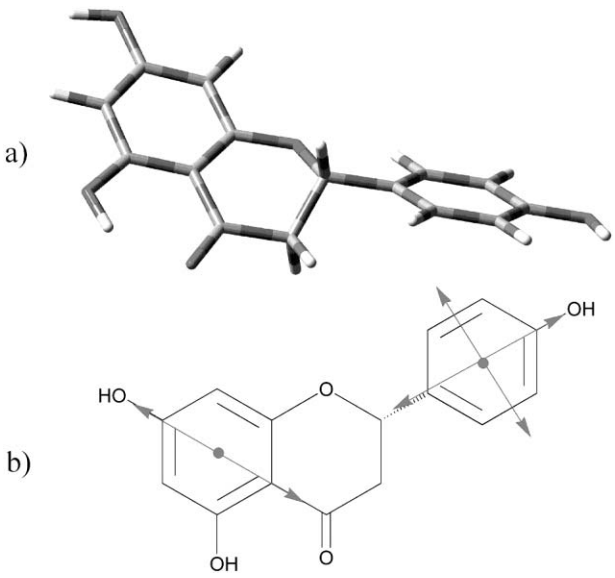

Fig. 2 (a) Structure of the lowest energy conformer of (*S*)-naringenin, found by MM2 calculations; (b) polarization directions (grey arrows) of the electric dipoles employed to represent the main allowed electronic transitions of the two chromophores.

The main geometrical characteristics of this structure are that: (1) the phenyl group linked to the flavanone ring is in equatorial position. The axial conformer is much higher in energy (*ca*. 1.6 kcal mol^{-1}) and therefore it is only slightly (6%) populated. The C3–C2–C1′–C2′ dihedral angle is 66°; (2) the 2,4,6-trihydroxyacetophenone portion of the molecule is almost completely planar. This is very important from the optical activity point of view, because contributions coming from intrinsically dissymmetric chromophores[17] (*i.e*. a twisted acetophenone moiety) which in principle can be large, can be safely ruled out; (3) the same MM calculations reveal the barrier to the rotation of the phenol group is of the order of 2 kcal mol^{-1}. This means that the phenol group can rotate at room temperature: this fact has important consequences as far as the optical activity calculations are concerned (*vide infra*).

DeVoe CD calculations

We shall briefly present here the most important features of the DeVoe model in order to understand how a practical calculation works and what are the necessary parameters. In the DeVoe model a molecule is considered to be composed of a set of subsystems, the chromophores; they are polarized by the external electromagnetic radiation and are coupled each other by their own dipolar oscillating fields. The optical properties (absorption, refraction, optical rotatory dispersion and circular dichroism) of the molecule under study can be calculated taking into account the interaction of the subsystems. Therefore, this treatment requires a division of the molecule into a set of subsystems that have to be suitably characterized. Then, each group is represented in terms of one (or more) classical oscillator(s); each oscillator represents an electric-dipole-allowed transition, defined by the polarization direction $\boldsymbol{e}_i$ and the complex polarizability $\alpha_i(\nu) = R_i(\nu) + \mathrm{i}I_i(\nu)$. $I_i(\nu)$ is obtainable from the experiment, *i.e*. from the absorption spectra of the compounds that can be considered good models of the subsystems, and $R_i(\nu)$ can be calculated from $I_i(\nu)$ by means of a Kronig–Kramers transform, ν is expressed in cm^{-1}. More often to simplify the calculation, a Lorentzian shape is assumed for an absorption band, so $I_i(\nu)$ and $R_i(\nu)$ can be obtained by simple analytical formulae which requires[18] the dipole strength, λ_{max} and the bandwidth. From the general formulation of the DeVoe model, retaining only the terms to first order in G_{12} (physically, this means considering that the electric dipole on the *i* chromophore is caused by the external e.m. field plus the dipolar fields of the other dipole polarized by the external field only) the following expression can be deduced in the case of two different chromophores having only one electrically allowed transition each which provides CD as a frequency function is: $\Delta\varepsilon(\nu) = 0.014\pi^2 N \boldsymbol{e}_1 X \boldsymbol{e}_2 \boldsymbol{R}_{12} G_{12} \nu^2 [I_1(\nu)R_2(\nu) + I_2(\nu)R_1(\nu)]$; $G_{12} = (1/r_{12})^3[\boldsymbol{e}_1 \cdot \boldsymbol{e}_2 - 3(\boldsymbol{e}_1\boldsymbol{e}_{12})(\boldsymbol{e}_2\boldsymbol{e}_{12})]$. Here $\boldsymbol{e}_1$, $\boldsymbol{e}_2$ are the unit direction vectors of the transition dipole moments of the first and second chromophore, respectively, $\boldsymbol{R}_{12}$ is the distance vector between them and r_{12} its modulus, G_{12} is the point-dipole–point-dipole interaction term. This expression gives rise to a couplet-like feature if the absorption maxima of the chromophores 1 and 2 are near in frequency ("quasidegenerate" coupled oscillator system). Of course, for several (say, *n*) oscillators we shall have, still to the same level of approximation (*i.e*. to first order) a sum over *n* of pairwise interactions, as that reported above. We are now able to carry out the DeVoe calculations of the CD spectrum of (*S*)-naringenin in the range 320–250 nm, *i.e*. we shall attempt to reproduce the Cotton effect present at 290 nm in the CD spectrum of (−)-**1**. To this end we shall employ as input geometry the structure of (*S*)-naringenin obtained from the MM2 calculations. The 290 nm absorption band of the substituted acetophenone chromophore will be described by a single oscillator, located in the centre of the benzene ring, polarized along the O=C (*y*) axis and carrying a dipole strength of 16 D^2 (to correctly reproduce the UV band). The phenol chromophore presents the transitions of simple 1,4-disubstituted benzenes:[19,20] the ^1L_a transition can be represented by a single oscillator, placed in the centre of the aromatic ring, polarized along the O–C direction and carrying a dipole strength of 8 D^2 (obtained from the experimental spectrum of phenol)[14] located at 213 nm. The ^{1}B transition can be described in terms of two[20] dipoles, placed in the centre of the aromatic ring, polarized along orthogonal directions lying in the aromatic plane (*e.g*. along the 1′–4′ and 2′–6′ directions, respectively)[21] and carrying a dipole strength of 30 D^2 each,[14] at 190 nm. However, the description of the phenol group, in particular of the ^{1}B transition, may present some difficulties, owing to the internal rotation of the phenol group itself. A problem of this type has been recently faced by Fleischhauer, Woody, Berova *et al*.[22] when they attempted the interpretation of the Cotton effects allied to the Soret transitions in some bis-porphyrin derivatives: so we shall follow their approach which takes into account two limiting situations. In the first one, which leads to the "effective transition moment" model, completely free rotation of the group is assumed: therefore the ^{1}B dipole with "transverse" polarization (*i.e*. 2′–6′ polarization) cannot give rise to exciton coupling with the 2,4,6-trihydroxyacetophenone chromophore dipole.[8c,22] Therefore, only the "longitudinal" dipoles (*i.e*. the ^1L_a dipole and the 1′–4′ component of the ^{1}B transition) are taken into account. This model produces a calculated 290 nm Cotton effect with $\Delta\varepsilon_{max}$ −2.3 to be compared to the experimental (−9.5) $\Delta\varepsilon_{max}$ value: *i.e*. for the (*S*) AC a negative Cotton effect at 290 nm is predicted, as experimentally found, even if, from a quantitative point of view, only a fraction of the experimental intensity is reproduced. In the alternative model proposed[22] the libration of the phenyl group is prevented, therefore the calculation will be carried out for the most stable equatorial conformer, taking into account both the components of the ^{1}B transition ("circular oscillator" model).[22] In this way, a calculated 290 nm Cotton effect with $\Delta\varepsilon_{max}$ −5.5 is obtained, *i.e*. a negative number, which is in a better quantitative agreement with the observed one. This good agreement between calculated and observed CD spectra establishes the safe configurational correlation (−)/(*S*). Furthermore, from this result one can also conclude that the rotation of the phenol residue is certainly not free, but this group is subjected to some kind of oscillation around the equilibrium position. Clearly, the Cotton effects at wavelengths longer than 320 nm and below 240 nm in the experimental spectrum of (−)-**1** cannot be reproduced simply because, for the reasons discussed above, no transitions on the

2,4,6-trihydroxyacetophenone chromophore have been taken into account in these two spectral ranges.

Ab initio calculation of the optical rotatory power of (−)-1

The *ab initio* calculation[10,11] of the optical rotatory power, for instance at the sodium D line, *i.e.* $[a]_D$, has become possible only very recently, mainly thanks to the extraordinary progresses of computational techniques: in this way one now can, at least in principle, assign the molecular AC by a comparison between the experimental rotation and the value predicted *ab initio*, assuming arbitrarily a certain AC, by means of some commercially available packages.[23–25] According to the general theory, [26] the OR (optical rotatory power) is obtained as specific rotation $[a]_\lambda$, for each angular frequency $\omega = 2\pi\nu = 2\pi c/\lambda = 2\pi c\bar{\nu}$ of the incident radiation, through the calculation of the optical parameter β, which is directly connected to the trace of the frequency-dependent electric-dipole–magnetic-dipole polarizability tensor $\boldsymbol{G'}$, *i.e.*

$$[a]_\lambda = 1.34229 \times 10^{-4}\beta\bar{\nu}^2(n^2+2)/3M$$

$$\beta = -\frac{1}{3\omega}\mathrm{Tr}[\mathrm{G}'(\omega)]$$

$$G'_{a\beta}(\omega) = -\frac{4\pi}{h}\sum_{j\neq 0}\frac{\omega}{\omega_j^2-\omega^2}\Im(\langle 0|\hat{\mu}|j\rangle\langle j|\hat{m}_\beta|0\rangle)$$

where the specific rotation is in unit of degrees [dm (g cm^{-3})]$^{-1}$, β in units of Bohr,[4] the radiation wavenumber in cm^{-1}; n is the refractive index of the medium, M the molar mass in g mol^{-1}; ω_j is the transition frequency from ground state $|0\rangle$ to excited state $\langle j|$, $\hat{\mu}$ and $\hat{m}$ the electric and magnetic dipole operators respectively. The assignment can be made if the theoretical result is fully reliable: from this point of view Stephens *et al.* established[10c] that a reliable *ab initio* calculation of the optical rotation requires the time dependent density functional theory (TDDFT) method (time dependent Hartree–Fock (TDHF) results being less accurate) with the hybrid B3LYP functional and the use of large basis sets, containing diffuse functions, *i.e.* the aug-cc-pVDZ basis set or larger. Such calculations also require the use, as input geometry, of a structure optimised at DFT/B3LYP/6-31G* level or higher. We decided to follow this protocol, reasoning that, since the experimental value is a small number ($[a]_D = -14.7$, ethanol) we need an accurate method, renouncing to our simplified approach.[27] The structure found by the MM2 calculations has been fully optimised at the DFT/B3LYP/6-31G* level. We found that the conformer with the phenyl group in equatorial position is 2.1 kcal/mol more stable than conformation with the phenyl group in axial position; so only the equatorial conformation was taken into account. Furthermore, taking into account the possible rotation of the phenol group around the $C_{1'}$–C_2 bond, a scansion of the potential energy surface (PES) has been carried out, varying the dihedral angle θ C_3–C_2–$C_{1'}$–$C_{2'}$ between 8° and 188°. The structure (Fig. 3) is similar to that found by MM2 calculations, but now $\theta = 80°$, *i.e.* the phenol plane is almost perpendicular to the heterocyclic ring.

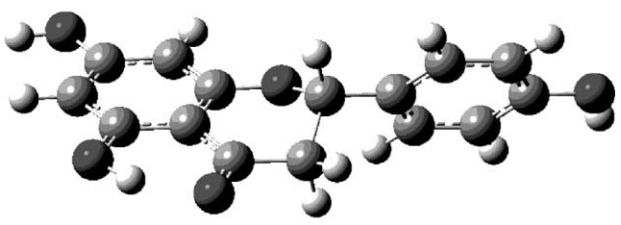

Fig. 3 Minimum energy structure of (*S*)-naringenin obtained by means of DFT/B3LYP/6-31G* calculations.

The conformer found constitutes the lowest point of a flat potential energy hole, as showed in Fig. 4.

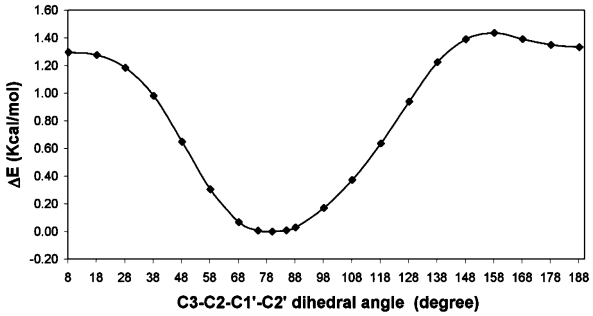

Fig. 4 DFT/B3LYP/6-31G* potential energy surface (PES) of (*S*)-naringenin as a function of the C_3–C_2–$C_{1'}$–$C_{2'}$ dihedral angle θ (for atom numbering see Chart 1).

Then the $[a]_D$ has been calculated at the TDDFT/B3LYP/aug-cc-pVDZ level, using, as input geometry, the above described minimum energy structure. A theoretical value of –238 is obtained, *i.e.* the correct sign of the optical rotation has been provided by this *ab initio* calculation, but the computed value is one order of magnitude larger than the experimental one. A possible explanation for such a large numerical difference between the experimental and the predicted value could be as follows: the flat potential energy hole we have found for (*S*)-naringenin guarantees that several different values of θ are easily accessible and therefore a correct prediction of the optical rotation requires an averaging over multiple orientations of the phenol ring with respect to the rest of the molecule, whilst we did a calculation for a single θ value. Thus, in order to appreciate the effect of the variation of this structural parameter upon the value of the optical rotation, we decided to calculate $[a]_D$ for several values of θ, the result is reported in Fig. 5: interestingly the optical rotation is negative for θ values between *ca.* 55° and *ca.* 143° and becomes positive and large outside this range, so one can easily realize that the effect of this parameter on the numerical outcome of the calculation can be significant. A similar problem has been recently faced by Stephens *et al.*, [28] who adopted a simple averaging algorithm: they calculated $[a]_D$ for a series of different θ angles, spanning 180°, and averaged the resulting values using Boltzmann statistics. We tried a slightly more complex approach. Firstly the experimental points of Figs. 4 and 5 have been interpolated using two sixth-order polynomial functions; $E(\theta)$ and $\mathrm{OR}(\theta)$ were so obtained. The Boltzmann averaged $[a]_D$ value was calculated, at $T = 298$ K, according to the following formula:[29]

$$[a]_D = \frac{\int_{8°}^{188°}\mathrm{OR}(\theta)e^{-E(\theta)/RT}d\theta}{\int_{8°}^{188°}e^{-E(\theta)/RT}d\theta}$$

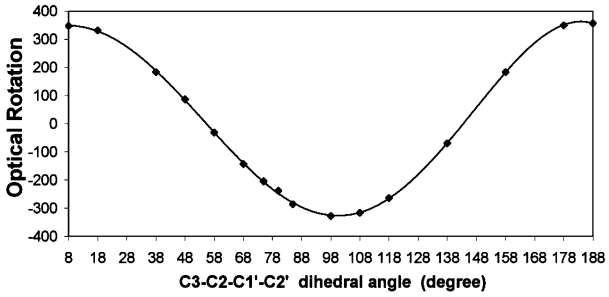

Fig. 5 Optical rotary power at the sodium D line, calculated at the TDDFT/B3LYP/aug-cc-pVDZ level, as a function of the C_3–C_2–$C_{1'}$–$C_{2'}$ dihedral angle θ.

In this way a $[a]_D$ −143 was obtained, *i.e.* a figure which is still an overestimate of the measure, but clearly demonstrates the importance of an efficient averaging algorithm to deal with the problem of the internal rotation of the phenol group. An alternative procedure corresponds to the assumption of free rotation around the $C_{1'}$–C_2 bond (*i.e.*, $E(\theta)$ = constant for all the θ values). In this way a value of $[a]_D = +14$ is obtained. Taking into account the discussion of the CD calculations made in the previous section, this second situation looks quite unrealistic and represents then an upper limit for $[a]_D$ which is actually not attainable, suggesting that, in any case, a negative optical rotatory power is related to (*S*)-naringenin.

Conclusions

In this work a non-empirical analysis of the chiroptical properties of (−)-**1** has been carried out. In fact, the CD spectrum has been interpreted by the DeVoe polarizability model and the optical rotary power has been calculated by the high level (TDDFT/B3LYP/aug-cc-pVDZ) *ab initio* method. Both the approaches establish the (−)/(*S*) correlation, confirming then the empirical assignment previously due to Gaffield.[7] However this investigation revealed another interesting point which deserves some discussion. With the former approach, one can easily and safely assign the absolute configuration, by means of a minimum computational effort. By contrast, in the latter case a difficulty arose, owing to the internal rotation of the phenol group. In fact, this motion forces to an averaging over multiple orientations of the phenol ring with respect to the rest of the molecule, making the overall treatment quite heavy. In other words, the present results seem to indicate that whilst the analysis of Cotton effects allied to electrically allowed transitions is nowadays a simple and powerful tool available to the experimental organic chemist, the *ab initio* calculation of the optical rotation may present, in the case of molecules with free internal motion(s), some problems which may be difficult to solve.

Experimental

HPLC and instrumental methods

(±)-Naringenin 95% was purchased from Sigma. The HPLC system consisted of a Varian 5060 liquid chromatograph with Valco 10 or 50 μL sample loops, a Jasco Uvidec III spectrophotometer operating at 292 nm and a Varian Data Jet 4400 integrator or Omniscribe recorder for fraction collecting. The polysaccharide derived column (250 mm × 4.6 mm) was Chiralcel OD-H (cellulose tris-3,5-dimethylphenylcarbamate) coated on 5μ silica gel from Daicel (Tokyo, Japan). Optical rotations were measured with a Jasco DIP-370 digital polarimeter, using a 10 cm microcell. Specific rotation $[a]_D^{22} = +15.8$ (c 0.30, EtOH) was measured for the first eluted peak of **1**, while the eluate from the second peak afforded an experimental $[a]_D^{22} = -14.7$ (c 0.36, EtOH). Absorption UV spectrum was recorded in ethanol on a UV-VIS Perkin Elmer Lambda 2S spectrometer. CD spectra were recorded on a Jasco J810 spectropolarimeter using 1 mm cell in ethanol.

Computational methods

All calculations have been carried out on a simple PC endowed with a single Pentium IV 2.2 GHz processor.

MM, CNDO and DeVoe computations. MM calculations were carried out using the MM2 force field.[16] CNDO/S-CI calculations have been carried out, using standard parameters,[15] by means of a routine kindly provided by the late Professor T. D. Bouman, University of Southern Illinois, USA. For a short description of the DeVoe coupled oscillator calculations see ref. 9*c*,*d*. Such calculations were performed by means of a program written by Hug and co-workers.[30] Both the programs are available, free of charge, from the authors of the present article.

***Ab initio* computations.** The geometry of (*S*)-naringenin has been fully optimized at DFT/B3LYP/6-31G* level using the Gaussian98 package, reaching a real minimum since no imaginary frequencies were found. The OR calculations have been carried out by means of time-dependent DFT/B3LYP/aug-cc-pVDZ method as available within Turbomole 5.6 package. [25]

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