JOC *Article*

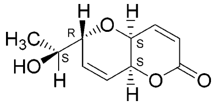
Assignment of the Absolute Configuration of (+)-Diplopyrone, the Main Phytotoxin Produced by *Diplodia mutila*, the Pathogen of the Cork Oak Decline, by a Nonempirical Analysis of Its Chiroptical Properties[†]

Egidio Giorgio,[‡] Lucia Maddau,[§] Emanuela Spanu,[§] Antonio Evidente,[*,||] and Carlo Rosini[*,‡]

Dipartimento di Chimica, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy, Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 1, 07100 Sassari, Italy, and Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli Federico II, Via Università 100, 80055 Portici (Napoli), Italy

rosini@unibas.it; evidente@unina.it

Received July 12, 2004

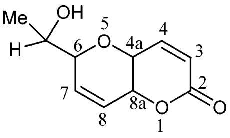


(+)-diplopyrone

The nonempirical assignment of the absolute configuration of (+)-diplopyrone, the main phytotoxin of *Diplodia mutila*, i.e., an endophytic fungus, widespread in Sardinian oak forests, and considered one of the main causes of cork oak decline, 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 methods indicate that (+)-diplopyrone is 6-[(1*S*)-1-hydroxyethyl]-2,-4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one, so the stereostructure of this important biomolecule is safely determined for the first time. A comparison of advantages and limitations of the two methods of analysis is also presented.

Introduction

(+)-Diplopyrone, (+)-**1**, is a new, optically active, phytotoxic monosubstituted tetrahydropyranopyran-2-one isolated as the main phytotoxin from the liquid culture filtrates of *Diplodia mutila* (Fr.) apud Mont., an amorph of *Botryosphaeria stevensii* Shoem.



diplopyrone-**1**

This microrganism is an endophytic fungus, widespread in Sardinian oak forests, and considered one of the main causes of cork oak (*Quercus suber* L.) decline.[1] The fungus can affect plants of different age, inducing symptoms very similar to those produced by tracheomycotic disease; thus, considering the large commercial use of cork, it causes heavy economic losses and environmental damages. Diplopyrone assayed at a 0.01−0.1 mg/mL concentration range causes necrosis and wilting on cork oak cuttings. On a nonhost plant, tomato, diplopyrone causes brown discoloration or stewing on the stem. Diplopyrone was characterized, using spectroscopic and chemical methods, as 6-[(1*S*)-1-hydroxyethyl]-2,4a,6,8a-tetrahydropyrano[3,2-*b*]pyran-2-one.[2] The absolute stereochemistry of the chiral secondary hydroxylated carbon in the exocyclic tail, determined by application of Mosher's method,[3] proved to be *S*.[2] The isolation and the identification of secondary metabolites could contribute to a better biological characterization of *D. mutila* providing a differentiation with respect to some other strains isolated from infected cypress and other oak species, which have been shown to produce different

---

[†] This paper is dedicated to Professor Piero Salvadori, a pioneer of chiroptical spectroscopy in Italy, on the occasion of his 70th birthday.

[‡] Università della Basilicata.

[§] Università di Sassari.

[||] Università di Napoli Federico II.

(1) Franceschini, A.; Corda, P.; Maddau, L.; Marras, F. *IOBC/wrps Bull.* **1999**, *22*, 5.

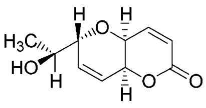
(2) Evidente, A.; Maddau, L.; Spanu, E.; Franceschini, A.; Lazzaroni, S.; Motta, A. *J. Nat. Prod.* **2003**, *66*, 313.

(3) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512 and references therein. Ohtani, I.; Kusumi, T.; Ishitsuki, M. O.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092. Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17.

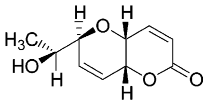
10.1021/jo0488255 CCC: $30.25

phytotoxic metabolites.[4] On the other hand, it is well-known that phytotoxins may be involved in pathogenesis and that microbial toxins may be used in the biological control of other pathogens which infect the same host plant as the toxin-producer microorganism. It is possible that diplopyrone produced by *D. mutila* could be used as antimicrobial substance against the strain of *D. mutila* infecting cypress and other oak species. Furthermore, the toxin could also be used to carry out experiments of genetic selection to obtain lines of host-plants resistant to the diseases.[5] The interesting potential practical applications of diplopyrone, produced in very low amounts in the fungal culture filtrates, require the availability of an efficient enantioselective synthesis and consequently the safe assignment of its absolute stereochemistry is an indispensable objective.

Therefore, the aim of this investigation is to arrive at a nonempirical assignment of the molecular absolute configuration (AC) of (+)-diplopyrone, (+)-**1**. The spectroscopic data collected in the previous investigation[2] established that (i) the two rings are connected by a *cis*-junction, (ii) the H4a and H8a hydrogen atoms lie on the same side and are opposite to the H6 hydrogen, and (iii) the absolute configuration at the sterocenter on the exocyclic tail is (*S*). As a consequence, (+)-**1** can only assume the structure I, i.e., 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one or the structure II, i.e., 6-[(1*S*)-1-hydroxyethyl]-2,4a(*R*),6(*S*),8a(*R*)-tetrahydropyrano[3,2-*b*]pyran-2-one.



structure I



structure II

We intend to establish the real stereostucture of (+)-**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[6] due to DeVoe.[7] In addition, we shall confirm the assignment reached above by means of a very new approach:[8,9] the *ab initio* calculation of the $[\alpha]_D$. In this way we arrive at a safe AC assignment of this interesting natural compound, and in addition, we can make a comparison of scope and limitations of these two different approaches.

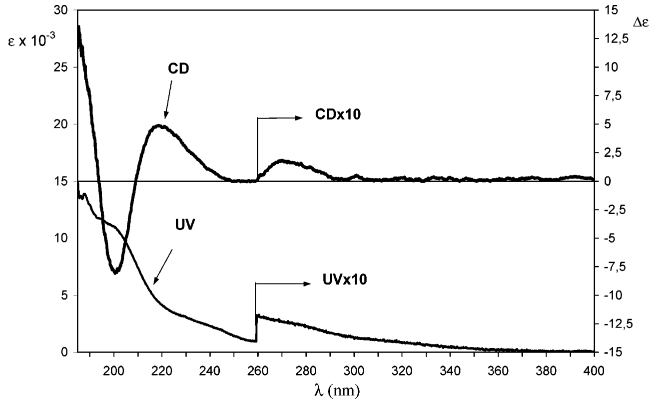


FIGURE 1. Absorption (UV) and circular dichroism (CD) spectra of (+)-**1** in trifluoroethanol.

Results and Discussion

$[\alpha]_D$, Absorption, and Circular Dichroism Spectra of (+)-Diplopyrone, (+)-1. Natural diplopyrone shows $[\alpha]_D$ +67.6 (*c* 0.25, $CHCl_3$) and +79 (*c* 0.1, MeOH). The absorption and circular dichroism (CD) spectra of (+)-**1**, measured in trifluoroethanol (this solvent has been used to gain transparency down to 185 nm and record also the positive Cotton effect at higher energies) between 400 and 185 nm, are collected in Figure 1.

The UV spectrum shows two main regions of absorption: a first, weak band is present at 270−275 nm (shoulder, ϵ ca. 200), followed by a more intense band at 200 nm (ϵ ca. 12000). Taking into account position and intensity, the first absorption band could be related to a $n \rightarrow \pi^*$ transition involving the unsaturated ester chromophore,[10] while the short wavelength region of absorption can result from transition involving both the α,β-unsaturated ester chromophore[11] as well as the olefinic group.[12] In the CD spectrum at least four different Cotton

---

(4) Evidente, A.; Sparapano, L.; Fierro, O.; Bruno, G.; Giordano, F.; Motta, A. *Phytochemistry* **1997**, *45*, 705.

(5) Evidente, A.; Motta, A. In *Bioactive Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis Group: London, 2001; p 473.

(6) Treatments of the coupled-oscillator model and its application to organic stereochemistry: (a) Mason, S. F. *Quart. Rev.* **1963**, *17*, 20. (b) Mason, S. F. Theory. In *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*; Snatzke, G., Ed.; Wiley and Sons: London, 1967; Chapter 4, p 71. (c) Gottarelli, G.; Mason, S. F.; Torre, G. *J. Chem. Soc. B* **1971**, 1349. (d) Charney, E. *The Molecular Basis of Optical Activity—Optical Rotatory Dispersion and Circular Dichroism*; Wiley and Sons: New York, 1979. (e) Mason, S. F. *Molecular Optical Activity and Chiral Discrimination*; Cambridge University Press: Cambridge, 1982. Qualitative exciton treatments leading to simple chirality rules can be found in: (f) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. (g) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983. (h) Nakanishi, K.; Berova, N. In *Circular Dichroism: Principles and Applications*; Nakanishi, K., Berova, N., Woody R. W., Eds.; VCH Publishers: New York, 1994; Chapter 13, p 361. (i) Nakanishi, K.; Berova, N. In *Circular Dichroism: Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds.; Wiley-VCH Publishers: New York, 2000; Chapter 12, p 337.

(7) (a) DeVoe, H. *J. Chem. Phys.* **1964**, *41*, 393; (b) **1965**, *43*, 3199. (c) Rosini, C.; Salvadori, P.; Zandomeneghi, M. *Tetrahedron: Asymmetry* **1993**, *4*, 545. (d) Superchi, S.; Giorgio, E.; Rosini, C. *Chirality* **2004**, *16*, 422.

(8) The first ab initio calculation of $[\alpha]_D$ has been carried out by Polavarapu. (a) Polavarapu, P. L. *Mol. Phys.* **1997**, *91*, 551. The method has been employed to assign the AC of a natural product for the first time by Kondru et al. (b) Kondru, R. K.; Wipf, P.; Beratan, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 2204. Stephens et al. performed a systematic study about scope and limitation of this method: (c) Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Phys. Chem. A* **2001**, *105*, 5356. For a recent review, see: (d) Polavarapu, P. L. *Chirality* **2002**, *14*, 768.

(9) Two very recent examples of assignment of absolute configuration by the ab initio calculation of $[\alpha]_D$ are: (a) Takeshi, K.; Miyako, F.; Toshiyuki, H. Tominari, C.; Junko, N.; Yukio, O.; Satoshi, H. *Chem. Pharm. Bull.* **2003**, *51*, 20. (b) Vogensen, S. B.; Greenwood, J. R.; Varmin, A. R.; Brehm, L.; Pickering, D. S.; Nielsen, B.; Liljefors, T.; Clausen, R. P.; Johansen, T. N.; Krogsgaard-Larsen, P. *Org. Biomol. Chem.* **2004**, *2*, 206.

(10) Saturated carboxyl acids show the $n \rightarrow \pi^*$ transition at about 200−210 nm (see, for instance: Murrel, J. N. *The theory of electronic spectra of organic molecules*; Methuen: London, 1963). It is reasonable to assume that the conjugation with a double bond will shift the position of this band toward the red.

(11) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983; p 43.

(12) Drake, A. F.; Mason, S. F. *Tetrahedron* **1977**, *33*, 937.

effects can be noticed: the lowest energy one is at about 275 nm ($\Delta\epsilon$ +0.25), followed by a second positive ($\Delta\epsilon$ +5) CD band at 220 nm and by a succession of two oppositely signed Cotton effects at 200 nm ($\Delta\epsilon$ −8) and 190 nm ($\Delta\epsilon$ +10). Considering that these two CD bands have the same intensity, with opposite signs and correspond to a region of intense absorption, they clearly constitute a CD "couplet",[13] deriving from the coupling of the electrically allowed transition on the above-quoted α,β-unsaturated ester chromophore and olefinic chromophore. As it is nowadays generally accepted, the presence in a CD spectrum of the so-called "exciton couplet" can be exploited[6] to carry out a nonempirical assignment of the molecular absolute configuration. In fact, the exciton (coupled oscillator) method constitutes nowadays a relatively simple, reliable, and powerful tool to assign the absolute configuration (AC) of organic molecules. To this end we shall use the DeVoe-coupled oscillator (or polarizability) model.[7]

DeVoe CD Model. DeVoe has developed[7a,b] a method for the calculation of the optical properties of molecular aggregates which is based on classical physics. This treatment does not constitute an empirical approach; in fact, it does not require any information about related systems having known structure. On the contrary, it makes possible the calculation of the CD spectrum as a frequency function, starting only from (vide infra) some spectroscopic information concerning the groups building the aggregate (location and polarization direction of the electrically allowed transitions of the groups which constitute the aggregate) and geometrical information (relative disposition of the groups within the aggregate, i.e., a known conformation once an absolute configuration has been assumed). The comparison between the predicted and experimental CD spectrum allows us to arrive nonempirically at the required configurational assignment. 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 to 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 $\mathbf{e_i}$ and the complex polarizability $\alpha_i(\nu) = R_i(\nu) + iI_i(\nu)$. $I_i(\nu)$ 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 transformation. 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 formulas which require[14] 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 electromagnetic field plus the dipolar fields of the other dipole polarized by the external field only) the following expression can be deduced (for two different chromophores having only one electrically allowed transition each) which provides CD as a frequency function: $\Delta\epsilon(\nu) = 0.014\pi^2 N\mathbf{e_1}X\mathbf{e_2}\boldsymbol{R_{12}}G_{12}\nu^2[I_1(\nu)R_2(\nu) + I_2(\nu)R_1(\nu)]$; $G_{12} = (1/r_{12})^3[\mathbf{e_1e_2} - 3(\mathbf{e_1e_{12}})(\mathbf{e_2e_{12}})]$. Here $\mathbf{e_1}$ and $\mathbf{e_2}$ 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, and ν is the frequency expressed in cm^{-1}. This expression gives rise to a couplet-like feature if the absorption maxima of the chromophore 1 and 2 are close in frequency ("quasidegenerate" coupled oscillator system). A correct application of the DeVoe method requires[6,7] the knowledge of the molecular conformation (structure of the single conformer, conformer distribution) to evaluate[7] all the necessary geometrical parameters ($\mathbf{e_1}$, $\mathbf{e_2}$, $\mathbf{R_{12}}$, G_{12}) and the knowledge of the characteristics of the electrically allowed transitions involved (i.e., polarization direction, location, allied dipole strength) to evaluate $I_i(\nu)$ and $R_i(\nu)$.

Geometrical Input Parameters. Molecular mechanics (MM2 force field)[15] calculations revealed that diplopyrone exists as two main conformers, the structure of which is represented in Figure 2, once the above-reported structure I has been arbitrarily assumed. Conformer (a) represents 80% of the population, while conformer (b) constitutes the remaining 20%, this conformer distribution being derived from a Boltzmann statistics, using the energies of the two conformers given by the MM2 calculations. It is interesting to note that this MM2 results are in keeping with those provided by the more accurate DFT/B3LYP/6-31G* calculation on a model compound of (+)-**1** (vide infra). The atomic coordinates so obtained allow to evaluate all the geometrical parameters necessary in the DeVoe calculation.[7d]

Spectroscopic Input Parameters. Often to simplify the calculation, a Lorentzian shape is assumed[7] for an absorption band, so $I_i(\nu)$ and $R_i(\nu)$ to be introduced in the DeVoe equations can be obtained by simple analytical formulas which require[14] the dipole strength, λ_{max}, and the bandwidth of the *i* absorption. The absorption and CD spectra in the 210−185 nm spectral region are due to the coupling of electrically allowed transitions located on the olefin and α,β-unsaturated ester chromophore, so we need suitable parameters to represent these transitions. As it is well-known[12] the olefin chromophore shows, in this spectral range, an allowed transition located at about 195 nm, polarized along the carbon−carbon double bond with ϵ_{max} of about 10000. So this transition will be represented by a single oscillator located in the center of the C=C bond, polarized along the C=C direction and

---

[13] The term "couplet" was introduced in 1971 (Haas, G.; Hulbert, P. B.; Klyne, W.; Prelog, V.; Snatzke, G. *Helv. Chim. Acta* **1971**, *54*, 491).

[14] Cech, C. L.; Hug, W.; Tinoco, I., Jr. *Biopolymers* **1976**, *15*, 131.

[15] (a) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. (b) Allinger, N. L.; Kok, R. A.; Imam, M. R. *J. Comput. Chem.* **1988**, *9*, 591. (c) Lii, J.-H.; Gallion, S.; Bender, C.; Wikstrom, H.; Allinger, N. L.; Flurchick, K. M.; Teeter, M. M. *J. Comput. Chem.* **1989**, *10*, 503.

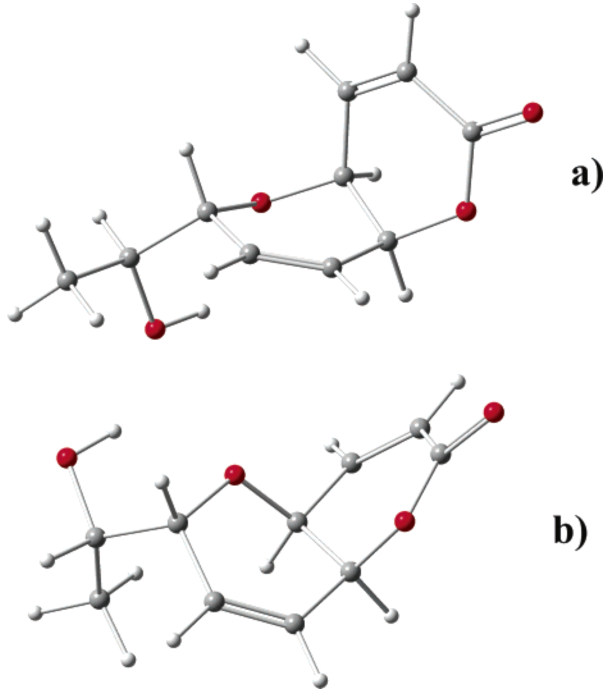


FIGURE 2. Structure of the (a) and (b) conformers of 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one, structure I, as found by the MM2 calculations.

carrying a dipole strength of 5 D^2, centered at 195 nm with a half-width of 3 kK. Since in the literature not much information is reported about the α,β-unsaturated ester chromophore, it is not easy to do a correct description of the corresponding oscillator (i.e., location, polarization, etc.). Therefore, we decided to do some CNDO/S-CI calculations using standard parameters[16] on methyl *trans*-butenoate, assumed as a good model of the α,β-unsaturated ester chromophore, using an input geometry obtained by MM2 computations. Within this scheme, the lowest energy, electrically allowed transition of the α,β-unsaturated ester chromophore can be correctly represented by a single HOMO−LUMO excitation (from MO17 to MO18). By using the c_i coefficients of the carbon p-orbitals, the vectorial bond order ($c_{\mu,17}c_{\nu,18}-c_{\mu,18}c_{\nu,17}$) of the transition, representing the transitional charge momentum along the carbon atoms μ and ν, was obtained, following Mason's[17] procedure which is based on the dipole-velocity formalism. This formalism guarantees the origin independency of the calculated rotational strengths when approximate molecular wave functions are used and provides a method to determine the correct location of the electric transition dipole moments. The calculated vectorial bond orders shows clearly that this transition involves mainly the C−C double bond, while the other bonds are involved to a very reduced extent. As a consequence, we can represent this electronic excitations in our DeVoe calculations employing a single oscillator located in the center of the C=C bond, polarized along the C=C direction and carrying a dipole strength of 5 D^2, centered at 195 nm with a half-width of 3 kK.

Results of the DeVoe Calculations and Absolute Configuration. Since we now possess all the necessary geometrical and spectroscopic parameters we are able to carry out the DeVoe calculations and produce a theoretical CD spectrum in the range 220−185 to be compared with the experimental one. It is interesting to note that the exocyclic alcoholic group does not absorb significantly in the region of interest being a saturated alcohol, so its presence can be safely disregarded. This makes enantiomeric structure I and structure II, quoted in the Introduction. Therefore, it will be sufficient carry out the calculations for only one of them, say 4a(*S*),8a(*S*),6(*R*), taking in to account the existence of two different conformers, a and b. Interestingly, both for conformer a and conformer b a negative couplet is predicted by our DeVoe calculations, the only difference being the intensity (−10; +10 for conformer a; −2.3; +2.3 for conformer b). So in Figure 3 the predicted (as a weighted average, using a Boltzmann statistic, of the CD spectra calculated for the single conformers) and the experimental absorption/CD spectra are reported: the excellent agreement (also from a quantitative point of view) clearly shows that (+)-diplopyrone possesses the 6-[(1*S*)-1-hydroxyethyl]-2,-4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one absolute configuration.

***Ab Initio* Calculation of the Optical Rotary Power of (+)-1.** The *ab initio* calculation[8,9] of the optical rotary power, for instance, at the sodium D line, i.e., $[\alpha]_D$, has become possible only very recently, mainly thanks to the extraordinary progress 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.[18−20] According to the general theory,[21] the OR is obtained as specific rotation $[\alpha]_\lambda$, for each

---

(16) Del Bene, J.; Jaffè, H. H. *J. Chem. Phys.* **1968**, *49*, 1221.

(17) Mason, S. F. *J. Chem. Soc., Chem. Commun.* **1973**, 239. Applications of this approach can be found in: Gottarelli, G.; Proni, G.; Spada, G. P.; Fabbri, D.; Gladiali, S.; Rosini, C. *J. Org. Chem.* **1996**, *61*, 2013. Tanaka, K.; Pescitelli, G.; Di Bari, L.; Xiao, T. L.; Nakanishi, K.; Armstrong, D. W.; Berova, N. *Org. Biomol. Chem.* **2004**, *2*, 48.

(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian98 and Gaussian03*; Gaussian Inc.: Pittsburgh, PA.

(19) Helgaker, T.; Jensen, H. J. Aa.; Joergensen, P.; Olsen, J.; Ruud, K.; Aagren, H.; Auer, A. A.; Bak, K. L.; Bakken, V.; Christiansen, O.; Coriani, S.; Dahle, P.; Dalskov, E. K.; Enevoldsen, T.; Fernandez, B.; Haettig, C.; Hald, K.; Halkier, A.; Heiberg, H.; Hettema, H.; Jonsson, D.; Kirpekar, S.; Kobayashi, R.; Koch, H.; Mikkelsen, K. V.; Norman, P.; Packer, M. J.; Pedersen, T. B.; Ruden, T. A.; Sanchez, A.; Saue, T.; Sauer, S. P. A.; Schimmelpfennig, B.; Sylvester-Hvid, K. O.; Taylor, P. R.; Vahtras, O. *DALTON, a molecular electronic structure program, Release 1.2*; 2001.

(20) Ahlrichs, R.; Bar, M.; Baron, H.-P.; Bauernschmitt, R.; Bocker, S.; Ehrig, M.; Eichkorn, K.; Elliott, S.; Furche, F.; Haase, F.; Haser, M.; Horn, H.; Hattig, C.; Huber, C.; Huniar, U.; Kattannek, M.; Kohn, A.; Kolmes, C.; Kollwitz, M.; May, K.; Ochsenfeld, C.; Öhm, H.; Schafer, A.; Schneider, U.; Treutler, O.; Arnim, M. v.; Weigend, F.; Weis, P.; Weiss, H. *TURBOMOLE, version 5.6*; Universitat Karlsruhe: Karlsruhe, Germany, 2002.

(21) (a) Rosenfeld, L. Z. *Phys.* **1928**, *52*, 161. (b) Condon, E. U. *Rev. Mod. Phys.* **1937**, *9*, 432. (c) Buckingham, A. D. *Adv. Chem. Phys.* **1967**, *12*, 107.

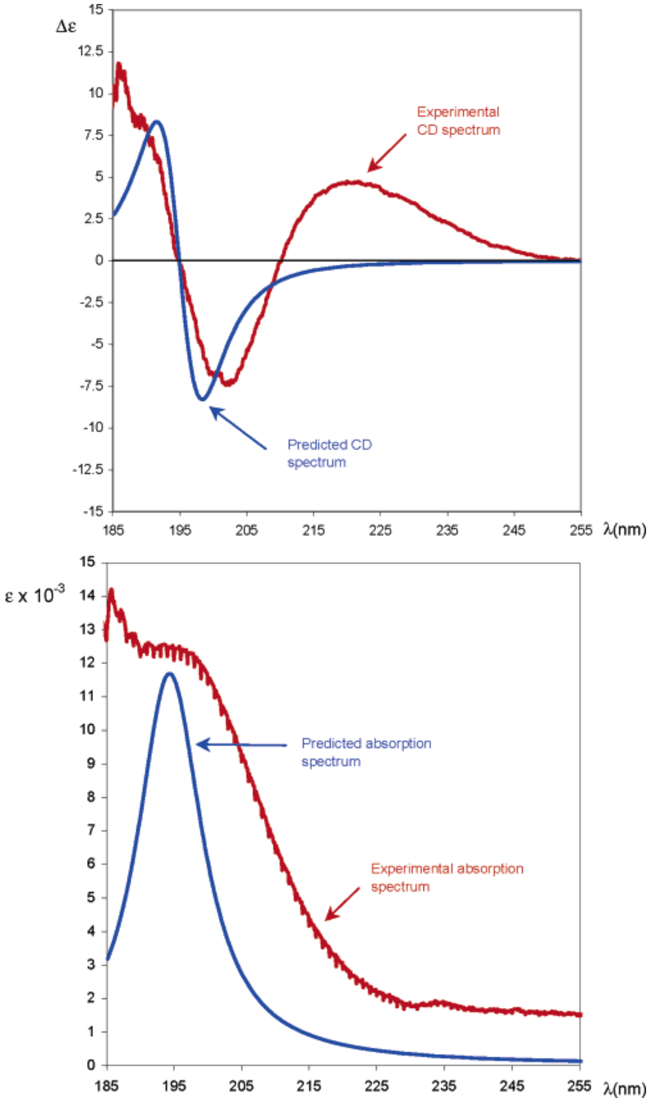


FIGURE 3. Lower curves: predicted (for 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one, structure I) and experimental absorption spectra of (+)-**1**. Upper curves: predicted (for 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one, structure I) and experimental CD spectra of (+)-**1**.

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.

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

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

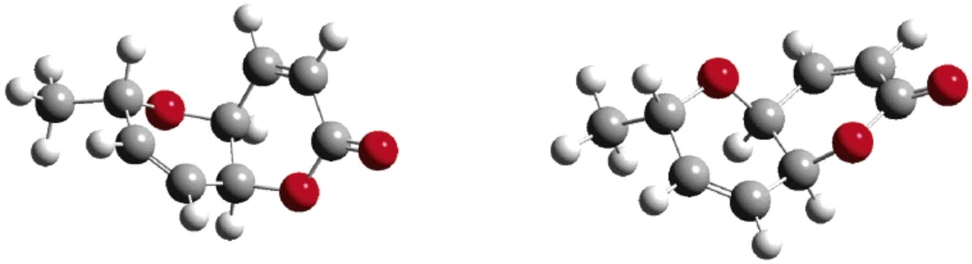
$$G'_{\alpha\beta}(\omega) = -\frac{4\pi}{h}\sum_{j\neq 0}\frac{\omega}{\omega_j^{\,2} - \omega^2}\mathscr{J}(\langle 0|\hat{\mu}_\alpha|j\rangle\langle 0|\hat{m}_\beta|0\rangle)$$

where the specific rotation is in deg [dm(g/cm^3)]$^{-1}$, β in (bohr),[4] the radiation wavenumber in cm^{-1}; n is the refractive index of the medium, M the molar mass in g/mol; ω_j is the transition frequency from ground state $|0\rangle$ to excited state $|j\rangle$, $\hat{\mu}$ 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[8c] 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 require also the use, as input geometry, a structure optimized at DFT/B3LYP/6-31G* level or higher. We decided to follow this protocol, reasoning that since (+)-**1** is a molecule with a reduced size the high level calculation of $[\alpha]_D$ suggested by Stephens *et al.*[8c] does not require a high computational effort. However, a significant difficulty could come, from this point of view, from the presence of the exocyclic alcoholic tail linked to C(6). In fact, this group is almost freely rotating (at least in methanol where a strong intramolecular hydrogen bond should not exist) so several different relative dispositions of the external tail with respect to the bicyclic part are easily accessible and therefore a correct prediction of the optical rotation requires an averaging over multiple orientations of the tail with respect to the rest of the molecule, making all the calculation procedures quite complex. A possible solution to this problem could come from a recent work of Grimme et al., who carried out[22] the ab initio calculation of the OR of (1*R*,2*R*)-1-chloroacetyl-2-fluorocyclohexane and other similar cyclic derivatives, concluding that " ...the optical rotation of these molecules is dominated by the local (1*R*,2*R*) configuration of the two substituents and that different ring and even axial/equatorial orientations play a less important role". Therefore, if the orientation of a substituent linked to a cyclic molecule gives a minor contribution to the OR value with respect to the local AC, we decided to carry out our $[\alpha]_D$ calculations, substituting the hydroxymethyl group linked to C6 with a methyl group, leaving C6 with the same absolute configuration. This structural approximation can be also justified taking into account that the carbon atom of the exocyclic tail linked to C(6) does not carry any strong chromophoric group while, by contrast, all of them are inserted in the bicyclic moiety of (+)-**1**, so the main contributions to the optical rotatory power must come from this molecular fragment and the contribution of the exocyclic tail to the OR at 589 nm is not substantial.[23] This approximation is useful not only in avoiding conformational problems but also because it makes enantiomeric the above-described structure I and structure II. Therefore it will be sufficient to carry out the calculations for only one of them, say 4a(*S*),8a(*S*),6(*R*), as we did before for the DeVoe prediction of the CD spectrum. This model compound exists (after conformational analysis at DFT/B3LYP/6-31G* level) as a mixture of two conformers with populations 78% and 22%, respectively. In the most populated conformer the methyl

---

[22] Grimme, S.; Bahlmann, A.; Haufe, G. *Chirality* **2002**, *14*, 793.

[23] As a matter of fact, the $[\alpha]_D$ of 2-butanol (a reasonable model of the C(6) tail) is 1.68 (ethanol) while 1-cyclohexyl-1-ethanol (a saturated fragment of our compound) shows 4.58 (ethanol), i.e., values which cannot certainly affect the OR of (+)-**1**, which is of the order of 80 units, in methanol.



Equatorial conformer (78%). Axial conformer (22%)

FIGURE 4. Structure of the equatorial and axial conformers of the 4a(*S*),8a(*S*),6(*R*)-model compound as obtained by means of DFT/B3LYP/6-31G* calculations.

group is equatorial while in the other it is axial, as shown in Figure 4.

The calculation of optical rotation at 589 nm has been carried out at TDDFT/B3LYP level, using the Sadlej basis set,[24] i.e., a basis set which is slightly larger than the aug-cc-pVDZ basis set suggested by Stephens et al.,[8c] and being purposely designed to correctly reproduce electric properties, should be particularly useful in calculating optical rotatory powers. As a matter of fact it provide excellent results in the calculation of the OR of (*S*)-propylene oxide.[25]

The predicted $[\alpha]_D$ value for the equatorial conformer is +35, while for the axial one we have +298. The average value, taking into account the relative populations of the two conformers, is +93, in good agreement with the experimental ones (using two different solvents we have $[\alpha]_D$ +67.6 (*c* 0.25, $CHCl_3$) and +79 (*c* 0.1, MeOH): this result confirms that the absolute stereostructure of (+)-**1** is given by 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano[3,2-*b*]pyran-2-one, structure I.

Conclusions

In this work a nonempirical analysis of the chiroptical properties of (+)-**1** has been carried out. In fact, the CD spectrum has been interpreted by means of the DeVoe polarizability model and the optical rotatory power has been calculated by the high level (TDDFT/B3LYP/Sadlej) ab initio method. Both the approaches independently support the (+)-6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a-(*S*)-tetrahydropyran[3,2-*b*]pyran-2-one configurational correlation. It is important to point out that this result has been achieved by two completely different approaches for the analysis of the chiroptical properties, so as Stephens et al.[26] pointed out when used the coupling of $[\alpha]_D$ and ECD calculation for the configurational assignment "... it is rather difficult that both types of calculations give simultaneously wrong results ...". Therefore, the configurational assignment herein proposed has to be considered as safe. In addition, this investigation revealed another interesting point which deserves some comments. With the former approach (i.e., the DeVoe method), one can easily and safely assign the absolute configuration, by means of a minimum computational effort. By contrast, in the latter case (i.e., the *ab initio* OR calculation) a difficulty arose, owing to the internal rotation around the bond connecting the tail to C(6). The presence of this motion would force to an averaging over multiple orientations of the exocyclic fragment with respect to the rest of the molecule, making quite laborious the overall treatment. We avoided this difficulty following a suggestion by Grimme et al.[22] and reasoning that the tail does not carry any strong chromophoric group while, by contrast, all of them are inserted in the bicyclic moiety of (+)-**1**, so the main contributions to the optical rotatory power must come from the bicyclic molecular fragment. Such an approximation saves a lot of computations, but it constitutes a reasonable assumption only when the mobile part, linked to a blocked, strongly absorbing system, is not chromophoric. In other words, the present results seem to indicate that the analysis of Cotton effects allied to electrically allowed transitions is nowadays a simple and powerful tool available to the experimental organic chemist, while the *ab initio* calculation of the optical rotation may present, in the case of molecules with free internal motion(s), some problems difficult to solve.

Experimental Section

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Absorption and CD spectra were recorded on a JASCO J600 spectropolarimeter at room temperature using trifluoroethanol solution in 0.1 and 1.00 mm cell. During the measurement the instrument was thoroughly purged with N_2.

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

MM, CNDO, and DeVoe Computations. MM calculations were carried out using the MM2 force field.[15] CNDO/S-CI calculations have been carried out, using standard parameters,[16] by means of a routine kindly provided by the late Professor T. D. Bouman, University of Southern Illinois. For a short description of the DeVoe coupled oscillator calculations see Rosini et al.[7c] and Superchi et al.[7d] Such calculations were performed by means of a program written by Hug et al.[27] Both of the programs are available, free of charge, from the authors of the present article.

Ab Initio Computations. The geometry of 4a(*S*)8a(*S*)6-(*R*) model compound has been fully optimized at the DFT/B3LYP/6-31G* level using the Gaussian98 package, reaching

---

[24] (a) Sadlej, A. J. *Collect. Czech. Chem. Commun.* **1988**, *53*, 1995. (b) Sadlej, A. J. *Theor. Chim. Acta* **1991**, *79*, 123.

[25] Giorgio, E.; Viglione, R. G.; Zanasi, R.; Rosini, C. *Chem. Phys. Lett.* **2003**, *376*, 452.

[26] Stephens, P. J.; McCann, D. M.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 7514.

[27] Hug, W.; Ciardelli, F.; Tinoco, I. *J. Am. Chem. Soc.* **1974**, *96*, 3407.

a real minimum since no imaginary frequencies were found. The OR calculations have been carried out by means of time-dependent DFT/B3LYP/Sadlej method as available within Turbomole 5.6 package.[20] Since Turbomole 5.6 uses the length formalism to calculate the OR values, the results are origin dependent, when small basis sets are used.[28] However, this dependence becomes negligible when extended basis sets are employed.[29] So the use of the Sadlej basis set, which is even slightly larger than the aug-cc-pVDZ set suggested by Stephens,[8c] guarantees, in practice, origin independence of the results.

Acknowledgment. Financial support from Università della Basilicata and MIUR-COFIN(Roma) is gratefully acknowledged. Contribution No. DISSPA 81.

JO0488255

---

(28) Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *J. Phys. Chem. A* **2000**, *104*, 1039.

(29) Grimme, S. *Chem. Phys. Lett.* **2001**, *339*, 380. (b) Grimme, S.; Furche, F.; Ahlrichs, R. *Chem. Phys. Lett.* **2002**, *361*, 321.