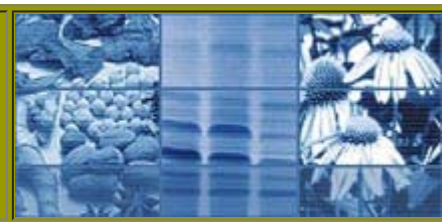


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Structure and Absolute Configuration of *Loliolide* Isomers Determined by Comparison of Calculated and Experimental CD Spectra

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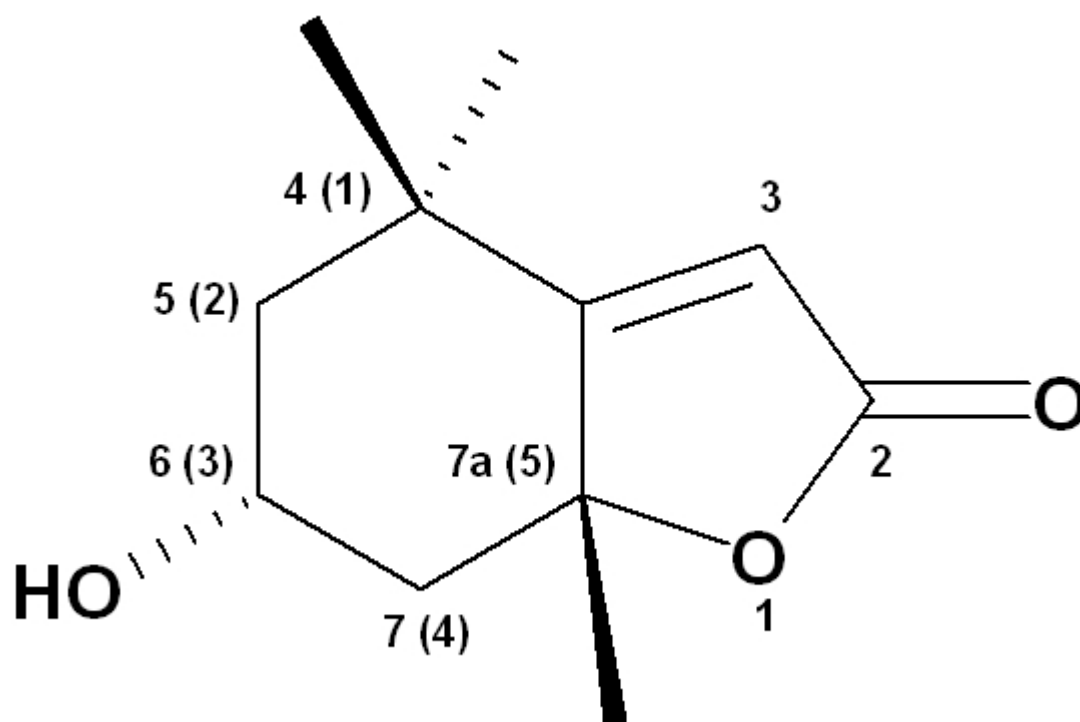
ABSTRACT

The circular dichroism spectra of the four possible configurations [(*R,R*), (*R,S*), (*S,R*), (*S,S*)] of loliolides are simulated on the basis of electronic excitation energies (wavelength) and rotational strengths *R* (nm) calculated by time dependent density functional theory using three density functionals (B3PW91, PBE0, B3LYP) and three basis sets [TZVP, SVP, 6-31G (d)]. All three basis sets give almost similar *l* and *R* with B3PW91 and B3LYP methods but with the PBE0 method, lower values of *l* and *R* were observed.

INTRODUCTION

The tetrahydro-2(4H)-benzofuranone *loliolide* (also calendin) has been isolated from a great variety of plants and essential oils, e.g. *Equisetum arvense* (Equisetaceae), *Clinopodium* sp. (Lamiaceae), *Salvia divinorum* (Lamiaceae), *Speranskia* (Euphorbiaceae), *Centaurea* (Asteraceae), many Fabaceae e.g. *Coronilla varia*, and *Lolium* (Poaceae). It was first isolated from *Lolium perenne* in 1964 [1]. Biosynthetically, this terpene results from degradation of carotenoids. It shows germination inhibitory [2] and also antitumor and immunosuppressive activity [3]. In this investigation, the hydrophilic extract of the aerial parts of *Pueraria lobata* (Kudzu Vine; Fabaceae) were phytochemically examined. Loliolide was found for the first time in this plant. The compound has been isolated by column chromatography on silica gel (hexane-ethyl acetate gradient) and subsequent preparative HPLC. Loliolide (C₁₁H₁₆O₃) has two chiral centers at C6 and C7a giving rise to two enantiomeric pairs of diastereoisomers, (6*R*, 7a*S*), (6*S*, 7a*S*) and (6*R*, 7a*S*), (6*S*, 7a*R*), respectively (Scheme 1). Actually, loliolide corresponds to the diastereoisomer with (*R,S*), (+)-loliolide and (*S,R*), (–)-loliolide relative configuration (Fig. 1A,1B). The compound with (*R,R*) relative stereochemistry is termed epiloliolide or isololiolide.

Scheme 1 Structure and IUPAC atom numbering of Loliolide (the atom numbering used in earlier publications is given in parentheses)



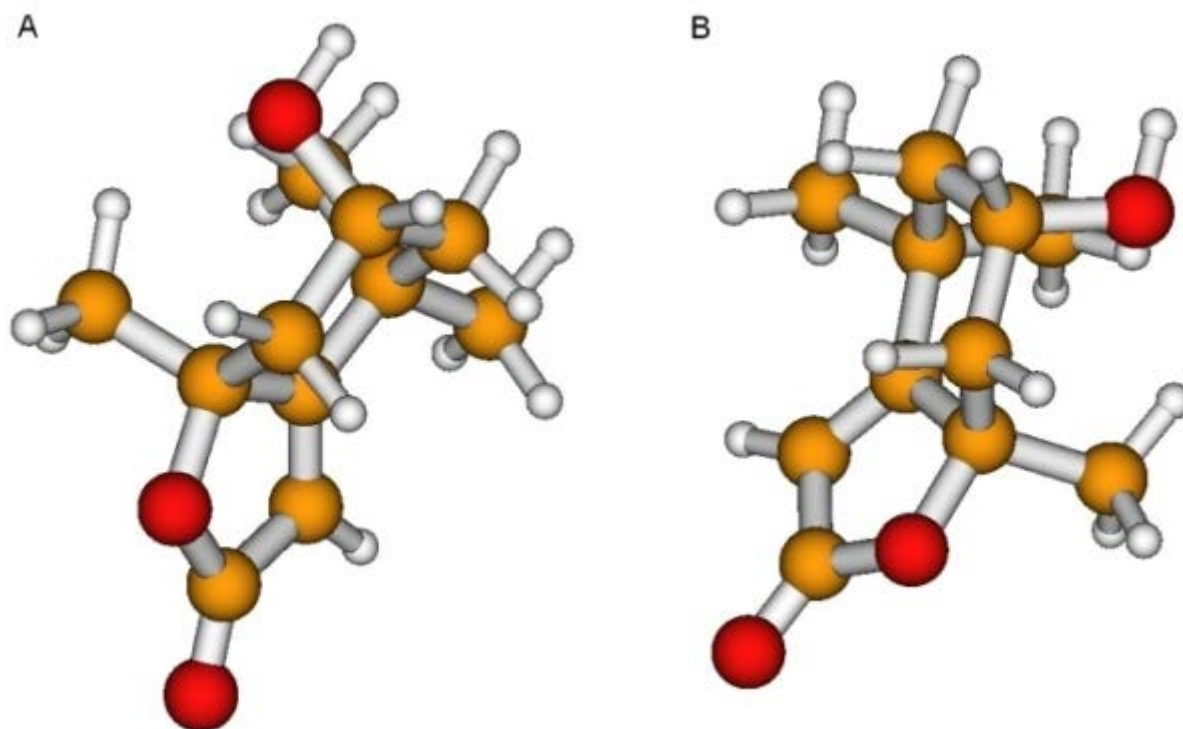
The absolute configuration of loliolide had been assigned chemically [4, 5] and with the help of the p-p* circular dichroism [6]. Since this tetrahydrobenzofuranone fragment is also contained in many other natural products, e.g. eremophilanoides, a detailed knowledge of its stereochemistry is of substantial interest. Theoretical calculations of chiroptical properties, such as optical rotatory dispersions, [7], vibrational circular dichroism, [8] and electronic CD spectra, [9,10] – especially by the time-dependent density functional theory (TDDFT) – have recently been applied successfully to the assignment of the absolute configuration of small- to medium-sized chiral organic molecules [11]. In continuation of our combined experimental and computational approach for the determination of the absolute configuration of natural products, we present here some preliminary results on the four possible stereoisomers of loliolide.

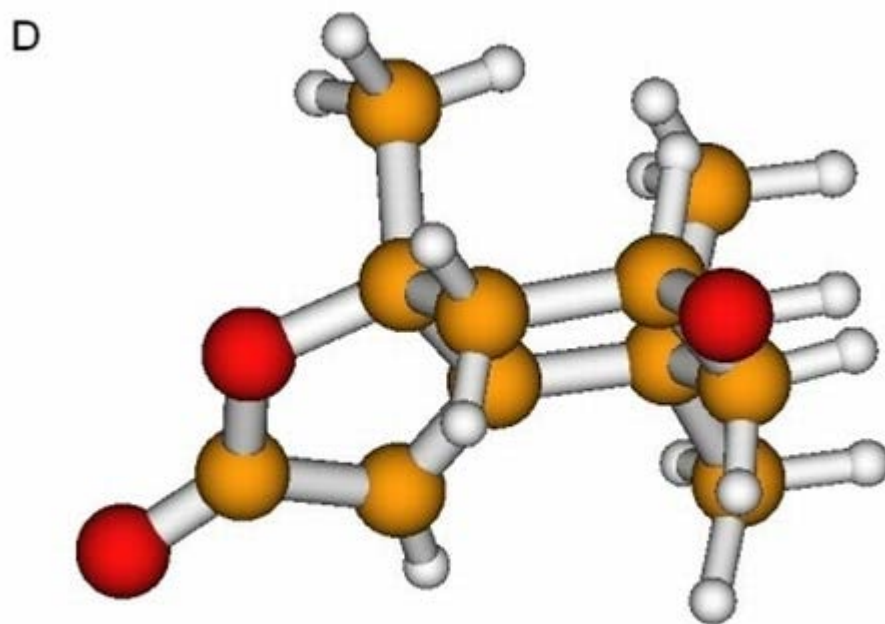
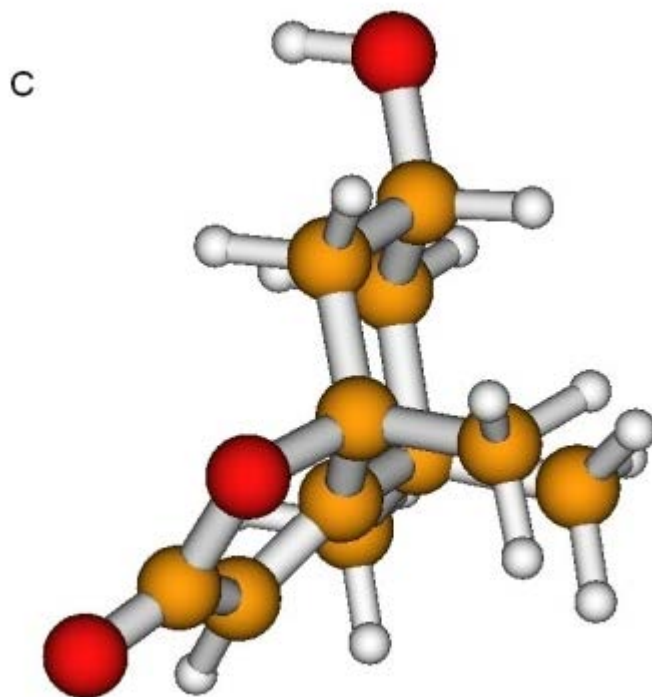
RESULTS and DISCUSSION

The calculated structure of loliolide is depicted in [Figure 1](#) and a

comparison of pertinent calculated geometric parameters with experimental X-ray data [3] is provided in Table 1. Mean absolute deviations (MAD) between calculated and experimental bond lengths (mean absolute deviation MAD = 0.015 Å), bond angles (MAD = 0.5°), and dihedral angles (MAD = 4.3°) are reasonably small and the two structures can be nearly perfectly overlaid (RMS = 0.016, atoms of the five-membered ring used for fitting). Calculated structures are depicted in Figure 1. The influence of the methods and basis set on calculated absorption wave lengths (1 / nm) and rotational strengths (R) is provided in Table 2.

Figure 1 Molden [20] plots of calculated [B3LYP/6-31G(d)] structures of the four possible loliolide stereoisomers, A: (6*R*7*aS*), and B: (6*S*7*aR*), C: (6*R*7*aR*), and D: (6*S*7*aS*) (For 3D representation, it might be necessary to install a molecular viewer)

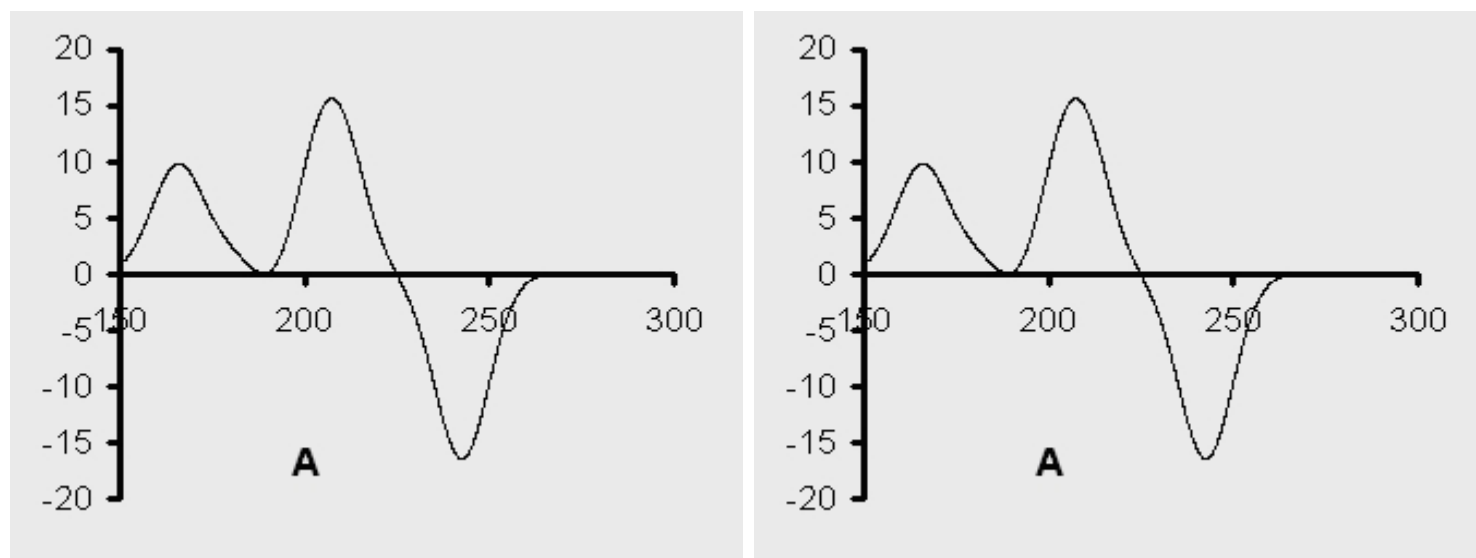




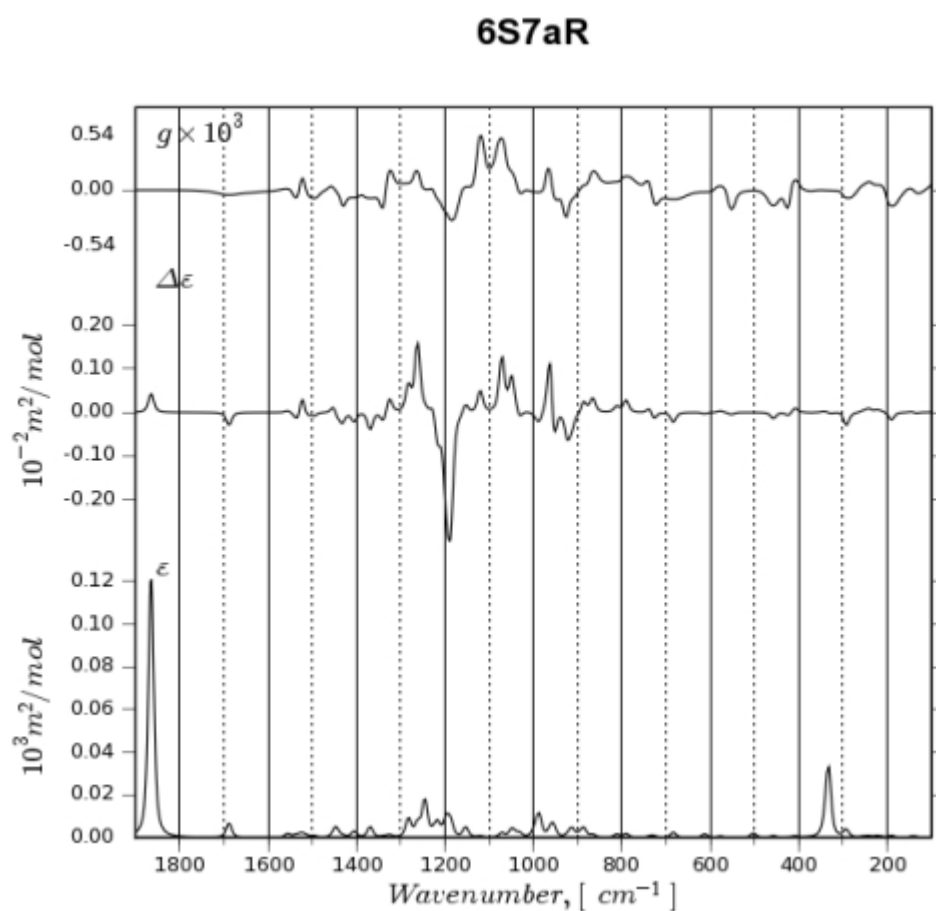
Experimental CD spectra apparently are only available for loliolide, i.e. the compound with (*R,S*) relative stereochemistry. In methanol, at $\lambda = 268$ nm a

weak positive ($De = +0.02$) and at $\lambda = 219$ nm a somewhat more pronounced negative ($De = -16.1$) peak in the CD spectrum has been quoted [6]. Interestingly, independent of the basis set and density functional used, our calculations on (6*S*,7*aR*) – loliolide do not predict such a weak positive CD effect; rather the first two electronic transitions should give rise to negative peaks in the CD spectrum. For instance, with B3PW91/TZVP a minimum at $\lambda = 242$ nm with $De = -16.4$, is predicted. In contrast, for the diastereoisomer with (*R,R*) relative configuration a weak long wavelength CD effect is predicted; its sign however, critically depends on the basis set used: with TZVP consistently $De > 0$ and with SVP and 6-31G(d) basis sets $De < 0$ result. Simulated CD spectra (B3PW91/TZVP) in methanol are depicted in [Figure 2](#). As an alternative to electronic CD spectra, vibrational circular dichroism has become a useful tool in the determination of absolute stereochemistry. Relevant portions of the calculated IR/VCD spectra of (6*S*,7*aR*) and (6*R*,7*aR*)loliolide diastereoisomers are shown in [Figure 3](#).

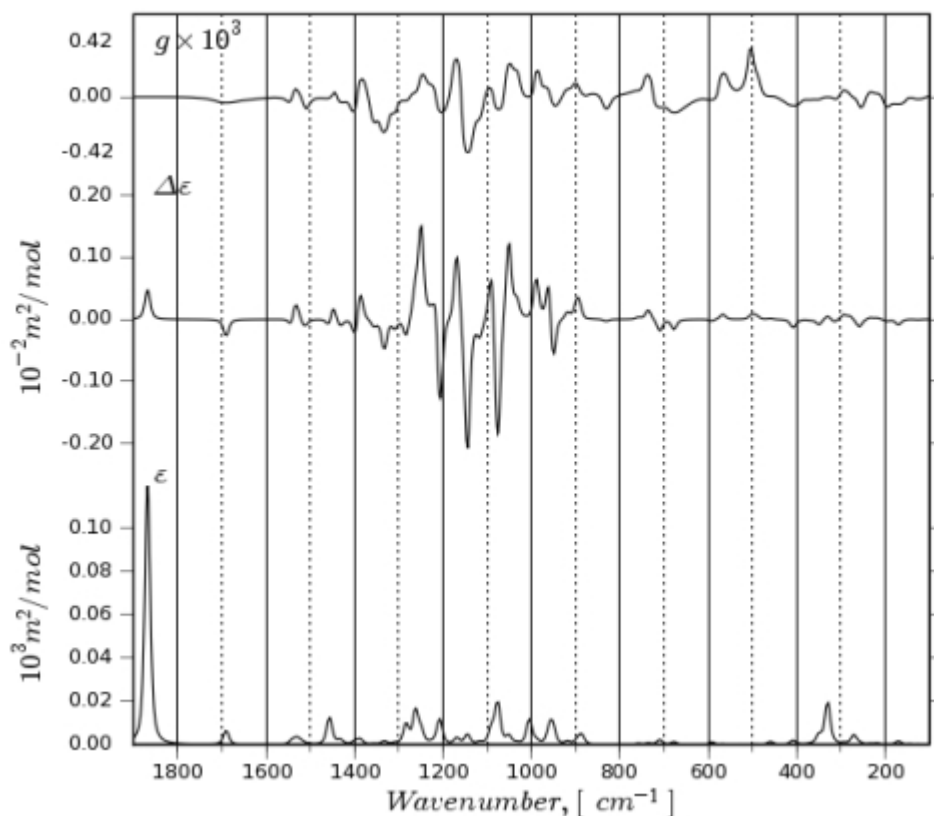
Figure 2 Simulated CD spectra (B3PW91/TZVP) in methanol for (6*S*,7*aR*) and (6*R*,7*aR*)loliolide diastereoisomers A and B, resp.



**Figure 3 Calculated IR and VCD spectra [B3LYP/6-31G(d)]
for (6*S*,7*aR*) and (6*R*,7*aR*)loliolide diastereoisomers**



6R7aR



CONCLUSIONS

Electronic and vibrational CD spectra for loliolide stereoisomers have been calculated by three different density functional methods (B3LYP, B3PW91, PBE0) and basis sets [TZVP, SVP, and 6-31G(d)]. Although by and large simulated spectra agree with experimental ones, several problems associated with the determination of absolute stereochemistry by comparison of calculated and experimental chiroptic properties exist. Work to resolve these discrepancies is in progress.

COMPUTATIONAL DETAILS

The starting structures were created with the Sybyl molecular modeling package [12], followed by B3LYP/6-31G(d) optimization [13,14]. Electronic excitation energies (wavelength λ /nm) and rotational strengths R (cgs) were calculated by TDDFT using three density functionals (B3PW91 [15], PBE0 [16], B3LYP) and three basis sets [TZVP, SVP, 6-31G(d)]. Bulk solvent effects (methanol) were approximated by the polarisable continuum model (IEF-PCM [17]). For all DFT calculations the Gaussian 03 program was used [18]. In the calculation of R the dipole length formula was used. CD spectra were simulated [19] as sums of Gaussians centered at the wavelengths of the responding electronic transitions and multiplied by the calculated rotational strength.

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Table 1 Comparison of Calculated [B3LYP/6-31G(d)] and experimental X-ray data for loliolide

	Calc	Exp		Calc	Exp
$r(\text{O1-C2})$	1.375	1.350	$a(\text{C3a-C4-C5})$	106.4	106.6
$r(\text{C2-C3})$	1.447	1.449	$a(\text{C4-C5-C6})$	116.6	117.1
$r(\text{C3-C3a})$	1.340	1.338	$a(\text{C5-C6-C7})$	112.5	112.3
$r(\text{C3a-C4})$	1.515	1.500	$a(\text{C6-C7-C7a})$	113.2	112.1
$r(\text{C4-C5})$	1.562	1.560	$a(\text{C7-C7a-O1})$	108.9	109.1
$r(\text{C5-C6})$	1.540	1.493	$t(\text{O2-C2-C3-C3a})$	-179.6	-177.4
$r(\text{C6-C7})$	1.535	1.543	$t(\text{C2-C3-C3a-C4})$	-168.9	169.1

<i>r</i> (C7-C7a)	1.542	1.530	t(C3-C3a-C4-C5)	121.0	124.0
<i>r</i> (C3a-C7a)	1.525	1.502	t(C3a-C4-C5-C6)	46.3	45.0
<i>r</i> (C7a-O1)	1.451	1.467	t(C4-C5-C6-C7)	-51.1	-51.0
<i>a</i> (C2-C3-C3a)	109.7	108.9	t(C5-C6-C7-C7a)	51.3	52.3
<i>a</i> (C3-C3a-C4)	129.2	128.7	t(C6-C7-C7a-O1)	-162.0	-162.4

Table 2 Calculated wavelengths and rotational strengths for loliolide diastereoisomers in methanol

(6<i>R</i>,7<i>aR</i>)									
B3PW91/TZVP		B3PW91/6-31G(d)		B3PW91/SVP		B3LYP/SVP		B3LYP/6-31G(d)	
l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)
243	0.62	247	-1.31	247	-0.86	248	-0.66	247	-1.01
231	-1.39	233	-0.35	234	-0.17	233	-0.52	231	-0.63
209	-81.65	206	-92.93	206	-101.69	207	-91.47	206	-83.60
203	40.10	200	49.53	201	60.39	200	47.46	199	37.64
(6<i>S</i>,7<i>aR</i>)									
PBE0/TZVP		PBE0/6-31G(d)		PBE0/SVP		B3LYP/TZVP			
l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)		
238	2.27	241	-0.06	242	0.47	243	1.34		
221	-3.73	221	-1.24	223	-0.95	230	-1.99		
207	-68.31	203	-80.55	203	-89.14	210	-72.80		
197	27.17	195	36.85	196	46.86	202	30.46		
(6<i>S</i>,7<i>aR</i>)									
B3PW91/TZVP		B3PW91/6-31G(d)		B3PW91/SVP		B3LYP/SVP		B3LYP/6-31G(d)	
l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)
243	-28.39	246	-14.39	246	-14.11	247	-10.44	246	-10.78
236	-3.06	235	-12.37	236	-12.18	234	-15.48	232	-15.92
209	26.89	205	13.47	206	19.61	206	12.09	206	7.97
199	24.57	198	14.82	198	9.40	198	11.00	198	15.99

PBE0/TZVP		PBE0/6-31G(d)		PBE0/SVP		B3LYP/TZVP	
l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)	l (nm)	R (cgs)
237	-16.68	240	-9.79	241	-9.29	242	-23.79
226	-27.48	224	-28.15	225	-27.47	234	-7.39
206	38.67	202	23.55	202	27.20	210	21.45
194	34.43	193	24.32	193	19.89	199	25.76