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Structural and Stereochemical Studies of a Tetralin Norsesquiterpenoid from *Ligularia kangtingensis*

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ABSTRACT A chiral tetralin norsesquiterpenoid, ligukangtinol, was isolated from the plant *Ligularia kangtingensis*. Its planar structure was determined by extensive analysis of spectroscopic data (MS, IR, and NMR), and (1*S*,3*R*) absolute configuration of the tetralin ring was established by TDDFT-ECD calculations of the solution conformers. Conformational analysis and ECD calculations proved that the semiempirical helicity rules of 6-hydroxytetralins correlating the ¹L_b Cotton effect and *P/M* helicity of the fused carbocyclic ring correctly predicts the absolute configuration and thus can be used for the configurational assignment of related substituted tetralin derivatives. *Chirality* 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: chiral tetralin; inverse tetralin helicity rule; TDDFT-ECD calculation; *Ligularia kangtingensis*; norsesquiterpenoid

INTRODUCTION

The genus *Ligularia* belongs to the Senecioneae tribe of the Compositae family, and comprise ca. 100 species in China.¹ More than 20 species in this genus have been used for a long time as folk remedies for their antibiotic, antiphlogistic, and antitumor activities.² Phytochemical research on many species of *Ligularia* have been reported by different groups, which established that this genus is an important source of sesquiterpens, many of which show antibacterial and antitumor bioactivities.^{3–5} *Ligularia kangtingensis* S.W. Liu is an indigenous plant in the Sichuan province of China living in the grass slopes at an altitude of near 4000 m.⁶ Herein we report the structure elucidation of a new chiral tetralin norsesquiterpenoid, ligukangtinol (**1**), isolated from *L. kangtingensis*.

MATERIALS AND METHODS

General and Instrumentation

Optical rotation was measured on a JASCO P-1020 polarimeter. The infrared (IR) spectrum was recorded on a Bruker (Billerica, MA) Tensor 27 FT-IR spectrometer (KBr). Electronic circular dichroism (ECD) spectra were obtained on a J-810 spectropolarimeter. High-resolution electrospray ionization, mass spectrometry (HRESI-MS) was measured on a Waters Q-TOF Premier. Nuclear magnetic resonance (NMR) (1D and 2D) spectra were recorded on Varian Unity 400/54 instrument and Bruker-AMX 500.

Plant Materials and Isolation

The whole plant of *Ligularia kangtingensis* was collected from Kangding County, Sichuan Province, People's Republic of China, in August, 2010. The plant was identified by Qin-Mao Fang, Institute of TCM Medicinal Resources and Cultivation, Sichuan Academy of Chinese Medicine Sciences. A voucher specimen (No. LK1008) was deposited in the School of Life Science and Technology, University of Electronic Science and Technology of China. Powdered whole plants of *L. kangtingensis* (5 kg) were extracted with 95% aq. ethanol under reflux. The extracts were concentrated in vacuo to yield a residue, which was suspended in H₂O and then extracted with petroleum ether and EtOAc, respectively. The petroleum ether extract (185 g) was chromatographed over a silica gel column (2000 g, 100–200 mesh, Qingdao marine chemical factory), eluted with a gradient solvent system [CHCl₃:MeOH (90:1–2:1)] to give 12 fractions (Fr.1–12). Fr. 4 (2.9 g) was chromatographed over a silica gel column (200–300 mesh, 60 g) eluted with

solvent systems of petroleum ether-acetone (5:1) and cyclohexane-EtOAc (4:1) to afford compound **1** (24 mg).

Ligukangtinol (1). Colorless oil; [α]_D²⁰ +46 (c 0.0035, MeOH); ECD (MeCN, λ [nm] ($\Delta\epsilon$), c = 1.17 × 10⁻²): 283 (0.03), 274sh (0.03), 221sh (0.42), 205 (0.68), 192 (–0.25); IR ν_{\max} (ethanol): 3421, 2926, 1739, 1593, 1462, 1281, 1087, 813 cm⁻¹; ¹H and ¹³C NMR in CDCl₃, see Table 1; HRESIMS *m/z* 275.1028 [M+K]⁺ (calcd for C₁₄H₂₀O₃K, 275.4099).

Computational Section

Mixed torsional/low mode conformational searches were carried out on the (1*S*,3*R*,9*S*) and (1*S*,3*S*,9*S*) diastereomers of **1** by means of the MacroModel 9.9.223 software (Schrödinger, New York, NY) using a Merck Molecular Force Field (MMFF) with an implicit solvent model for chloroform. Geometry reoptimizations at B3LYP/6-31G(d) in vacuo and B3LYP/TZVP levels of theory applying a Polarizable Continuum Model (PCM) solvent model for acetonitrile followed by time-dependent Density Functional Theory (TDDFT) calculations using various functionals (B3LYP, BH&HLYP, PBE0) and TZVP basis set were performed with the Gaussian 09 package.⁷ ECD spectra were generated as the sum of Gaussians with 3000 cm⁻¹ half-height width (corresponding to ca. 13 nm at 210 nm), using dipole-velocity computed rotational strengths.⁸ Boltzmann distributions were estimated from the ZPVE corrected B3LYP/6-31G(d) energies in the gas phase calculations and from the B3LYP/TZVP energies in the solvated ones. The MOLEKEL software package was used for visualization of the results.⁹

RESULTS AND DISCUSSION

Ligukangtinol (**1**) was isolated as colorless oil with specific rotation [α]_D²⁰ = +46 (c 0.0035, MeOH). Its molecular formula was determined as C₁₄H₂₀O₃ on the basis of HRESI-MS peak of [M+K]⁺ at 275.1028 (calcd. 275.4099), indicating five

Dedicated to Prof. Dr. Sándor Antus on the occasion of his 70th birthday.
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TABLE 1. ^1H and ^{13}C NMR data (400/100 MHz) for compound **1** in CDCl_3

Position	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{a}}$ (multi, J in MHz)
1	70.6 (CH)	4.67 (1H, br s)
2	25.7 (CH_2)	1.50 (1H, d, 13.0) 2.41 (1H, dt, 13.0, 4.0)
3	30.8 (CH)	2.12 (1H, m)
4	34.7 (CH_2)	2.66 (1H, d, 18.0) 2.95 (1H, dd, 18.0, 6.9)
5	120.7 (C)	—
5a	138.5 (C)	—
6	153.2 (C)	—
7	112.8 (CH)	6.65 (1H, d, 8.0)
8	128.2 (CH)	6.96 (1H, d, 8.0)
8a	127.0 (C)	—
9	36.0 (CH)	1.59 (1H, m)
10	62.8 (CH_2)	3.19 (1H, d, 12.0) 3.47 (1H, dd, 12.0, 3.6)
11	18.7 (CH_3)	1.26 (3H, d, 7.6)
12	10.7 (CH_3)	2.14 (3H, s)

degrees of unsaturation. The IR spectrum showed absorption bands characteristic of hydroxyl groups (3421 cm^{-1}) and a benzene ring ($1593, 1462\text{ cm}^{-1}$).

The ^1H NMR data (CDCl_3 , Table 1) showed distinct signals for two methyls [δ_{H} 2.14 (3H, s); δ_{H} 1.26 (3H, d, $J=7.6\text{ Hz}$)] and two *ortho* aromatic protons [δ_{H} 6.65 (1H, d, $J=8.0\text{ Hz}$); δ_{H} 6.96 (1H, d, $J=8.0\text{ Hz}$)]. The ^{13}C NMR data (CDCl_3 , Table 1) revealed 14 carbon resonances that were distinguished via DEPT and HMQC data to be two methyls, three methylenes (including one oxygenated carbon), five methines (including one oxygenated and two aromatic carbons), and four aromatic quaternary carbons (including one oxygenated carbon). The data mentioned above accounted for 4 out of 5 degrees of unsaturation, indicating one additional ring in the structure of **1**.

The planar structure for **1** was constructed by 2D NMR data (Fig. 1). ^1H - ^1H COSY data indicated the existence of a proton spin system of $-(\text{O})\text{CH}-\text{CH}_2-\text{CH}(\text{CH}(\text{CH}_3)\text{CH}_2\text{OH})-\text{CH}_2-$. On the other hand, the ^1H and ^{13}C NMR data (Table 1), together with the correlation between the two aromatic protons in ^1H - ^1H COSY, showed the presence of one 1, 2, 3, 4-tetra-substituted phenyl ring. These two structural fragments mentioned above, along with other isolated groups, could be connected by further interpretation of HMBC data. The existence of two ring system was confirmed by the HMBC correlations of $\text{H}_3-12/\text{C}-5\text{a}$, C-5, C-6; H-1/C-5a, C-8; $\text{H}_2-4/\text{C}-5\text{a}$, C-5, C-8a. From the data above, together with the chemotaxonomy of genus *Ligularia*, a phenolic norsesquiterpenoid skeleton, the same as that of ligudentatol,¹⁰ could be deduced for compound **1**.

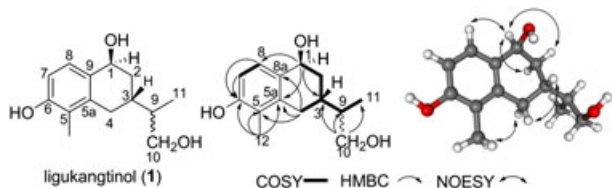
In HMBC spectrum, correlations between downfield aromatic carbon (δ_{C} 153.2) and H-7 (δ_{H} 6.65), H-8 (δ_{H} 6.96), H-12 (δ_{H} 2.14) indicated the position of the phenolic OH

(positive color test with ethanolic ferric chloride) as *ortho* to C-5 methyl group. The other two hydroxyl groups, which were deduced from the HRESI-MS and NMR data, were placed at C-1 and C-10 based on the HMBC data. Thus, the planar structure of **1** was determined as shown in Figure 1 and it was named ligikangtanol.

The relative configuration of **1** was determined by nuclear Overhauser effect spectroscopy (NOESY) measurement and $^3J_{\text{H,H}}$ data supported by computational conformational analysis. The 1-H proton showed NOE correlations with 8-H and both 2-H protons and it has a broad singlet signal in the ^1H -NMR spectrum suggesting its equatorial orientation. Thus, the 1-OH group adopted an *axial* orientation, by which it can reduce the unfavorable van der Waals repulsion with the *peri* 8-H proton. The absence of characteristic NOE correlations between 1-H and 3-H suggested the *trans* relative configuration of the 1-H and 3-H, which was also confirmed by conformational analysis and ECD calculations of *trans*-(1*S*,3*R*,9*S*)-**1** and the epimeric *cis*-(1*S*,3*S*,9*S*)-**1**. The conformational analysis of *trans*-(1*S*,3*R*,9*S*)-**1** using an initial MMFF conformational search followed by reoptimization at the B3LYP/TZVP level of theory with PCM solvent model for acetonitrile revealed that in all the conformers the 1-OH is *axial*, while the C-3 substituent is equatorial. A similar analysis of the *cis*-(1*S*,3*S*,9*S*)-**1** showed that all the conformers but one had 1,3-*cis* diaxial orientation for the 1- and 3-H protons, which should give NOE correlations between the latter protons. The β -4-H had NOE correlation with the 3-H and it has 18.0 and 6.9 Hz $^3J_{\text{H,H}}$ values for the geminal and vicinal couplings, respectively. The vicinal coupling of the α -4-H could not be resolved ($<1\text{ Hz}$) and it gave NOE with the C-5 methyl group. These data indicated that the C-4 methylene group is shifted upward, which distorted the half-chair conformation of the fused carbocyclic ring and moved the β -4-H to a quasi-axial position. This distortion also moves the 3-H toward the equatorial orientation, which is reflected in the small $^3J_{2-\text{Hax},3-\text{H}}$ coupling constant (4.0 Hz). The lack of NOE correlation between α -2- H_{ax} and α -4-H supported that the half-chair conformation is distorted by flipping C-4 upward. Interestingly, the conformational analysis of *cis*- or *trans*-**1** did not indicate any distortion of the half-chair conformation even using the PCM solvent model. Due to the relatively free rotation of the C-3 substituent, the relative configuration of the C-9 chirality center could not be assigned and it was arbitrarily defined as (9*S*) for the conformational analysis.

Compound **1** contains a substituted chiral tetralin chromophore, the absolute configuration of which can be determined by the $^1\text{L}_b$ band Cotton effect (CE) of the fused benzene chromophore in the ECD spectrum.¹¹ In chiral tetralin derivatives having no substituents on the fused aromatic ring, the $^1\text{L}_b$ CE is determined mainly by the *P* or *M* helicity of the fused nonaromatic chiral ring defined by the $\omega_{\text{C-8a,C-1,C-2,C-3}}$ torsional angle, which in turn is directed by the absolute configuration of the fused ring (Fig. 2). Snatzke and Ho developed a so-called helicity rule for the benzene chromophore of chiral tetralin derivatives (Fig. 2),¹² according to which if the benzene ring is not further substituted, *P* helicity of the nonaromatic ring leads to a positive CE within the $^1\text{L}_b$ band and, vice versa, *M* helicity is manifested in a negative one.

Snatzke et al.^{13,14} also showed that achiral substituents of the benzene ring with large spectroscopic moment (e.g. $q_{\text{OMe}} = +21 [(\text{cm mol})/\text{L}]^{-1/2}$)¹⁵ in specific positions inverted the helicity rule. This inversion was attributed to the change of the direction of the sum spectroscopic moment¹⁵⁻¹⁸ vector

**Fig. 1.** Structure and key 2D NMR correlations of ligikangtanol (**1**).

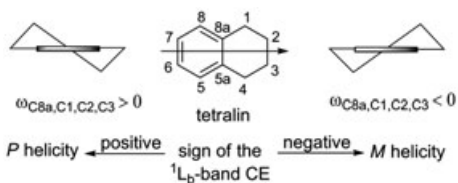


Fig. 2. Snatzke's helicity rule or correlation between the sign of the second sphere contribution of tetralin and the 1L_b band CE. The arrow indicates the direction of the overall spectroscopic moment. *P* and *M* helicity refer to the absolute conformation of the nonaromatic ring.

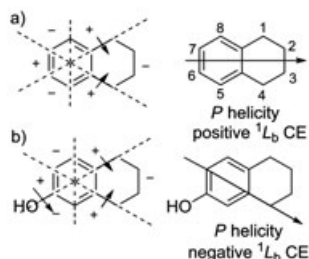
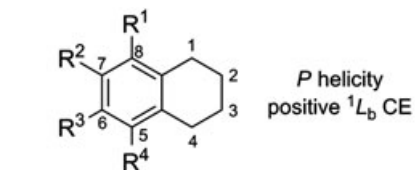


Fig. 3. Polarization diagram of the 1L_b band, direction of the overall spectroscopic moment and helicity rule of (a) tetralin, (b) 6-hydroxytetralin.

which gives the electric transition moment vector (μ); i.e., the translation of the electron charge during the transition. In Snatzke's terminology, achiral ring substituents can induce the inversion of the original helicity rule, which is the consequence of rotating the electric transition moment by approximately 30° . Figure 3a shows a polarization diagram of the tetralin chromophore in which the addition of the spectroscopic moments oriented the electric transition moment along the direction of the C-2 axis of the chromophore, which gives a positive 1L_b -band CE for *P* helicity of the nonaromatic ring

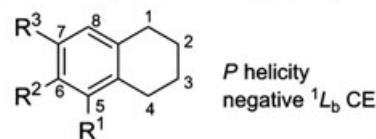


tetralin: $R^1, R^2, R^3, R^4 = H$

5,8-dimethoxytetralin: $R^1, R^4 = OMe, R^2, R^3 = H$

6,7-dimethoxytetralin: $R^1, R^4 = H, R^2, R^3 = OMe$

5,6,7-trimethoxytetralin: $R^1 = H, R^2, R^3, R^4 = OMe$



6-methoxytetralin: $R^1, R^3 = H, R^2 = OMe$

5,6-dimethoxytetralin: $R^1, R^2 = OMe, R^3 = H$

5,7-dimethoxytetralin: $R^1, R^3 = OMe, R^2 = H$

Fig. 4. Effect of achiral ring substituents of large spectroscopic moment (e.g., OMe) on the tetralin helicity rule.

(helicity rule of unsubstituted tetraline). In contrast, when tetralin has only one methoxy or hydroxy group at C-6, the sum of the spectroscopic moments rotates the electric transition moment by approximately 30° , which leads to a sign inversion as shown in Figure 3b. It was demonstrated that 5,8- and 6,7-disubstituted and 5,6,7-trisubstituted tetralins follow the same helicity rule as the unsubstituted tetralin, while 6-monosubstituted, and 5,6- and 5,7-disubstituted tetralins obey the inverse one (Fig. 4).^{13,14}

Since the C-5 methyl group has low spectroscopic moment and it does not interfere with the helicity rule, compound **1**

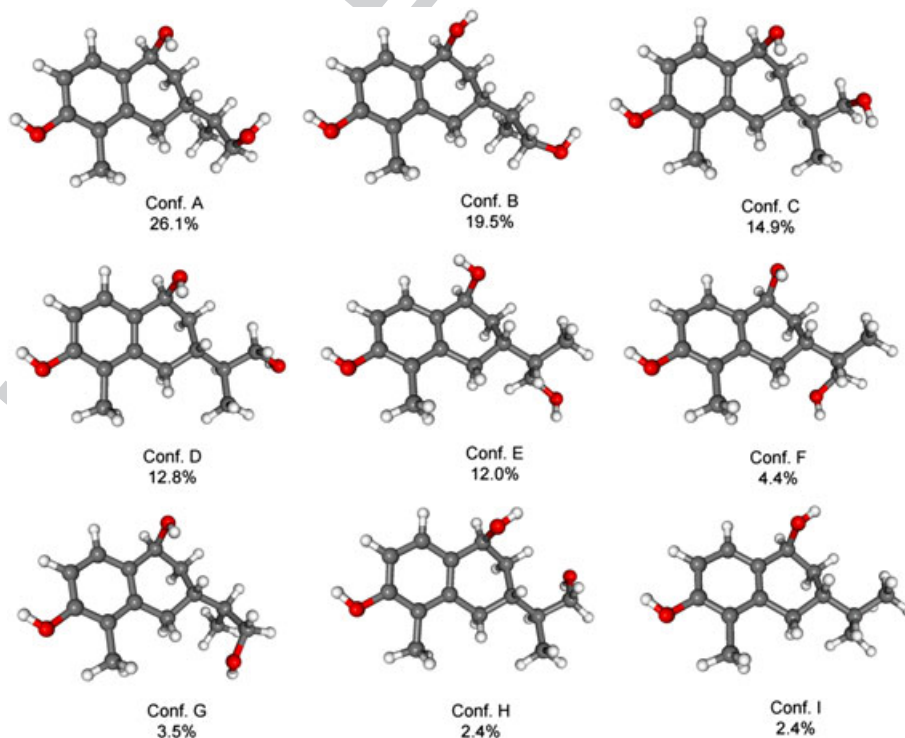


Fig. 5. Low-energy conformers (>1%) of *trans*-(1*S*,3*R*,9*S*)-**1** optimized at B3LYP/TZP level of theory with PCM model for MeCN.

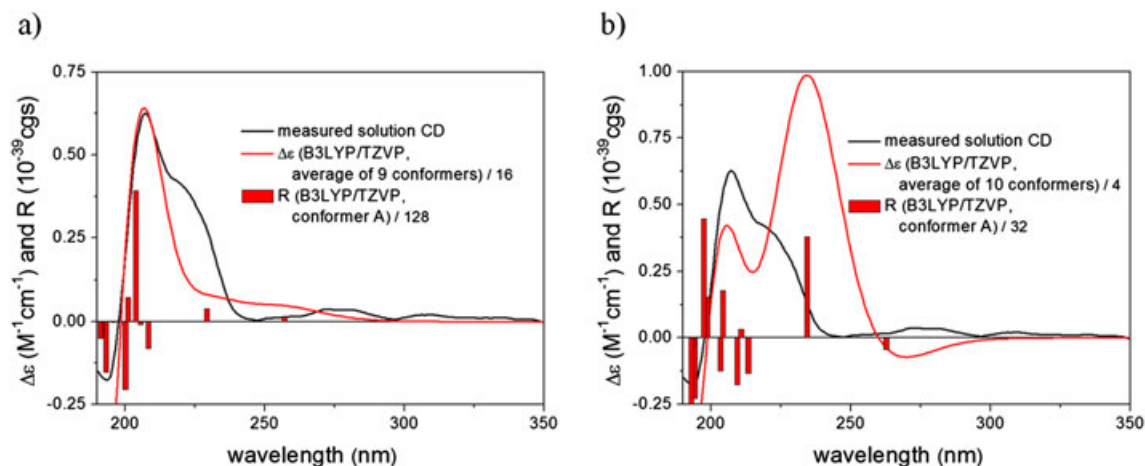


Fig. 6. Experimental and B3LYP/TZVP ECD spectra of (a) *trans*-(1S,3R,9S)-**1** and (b) *cis*-(1S,3S,9S)-**1** obtained as the Boltzmann-weighted average of the computed low-energy solution conformers. Bars represent rotational strength of the lowest-energy conformer.

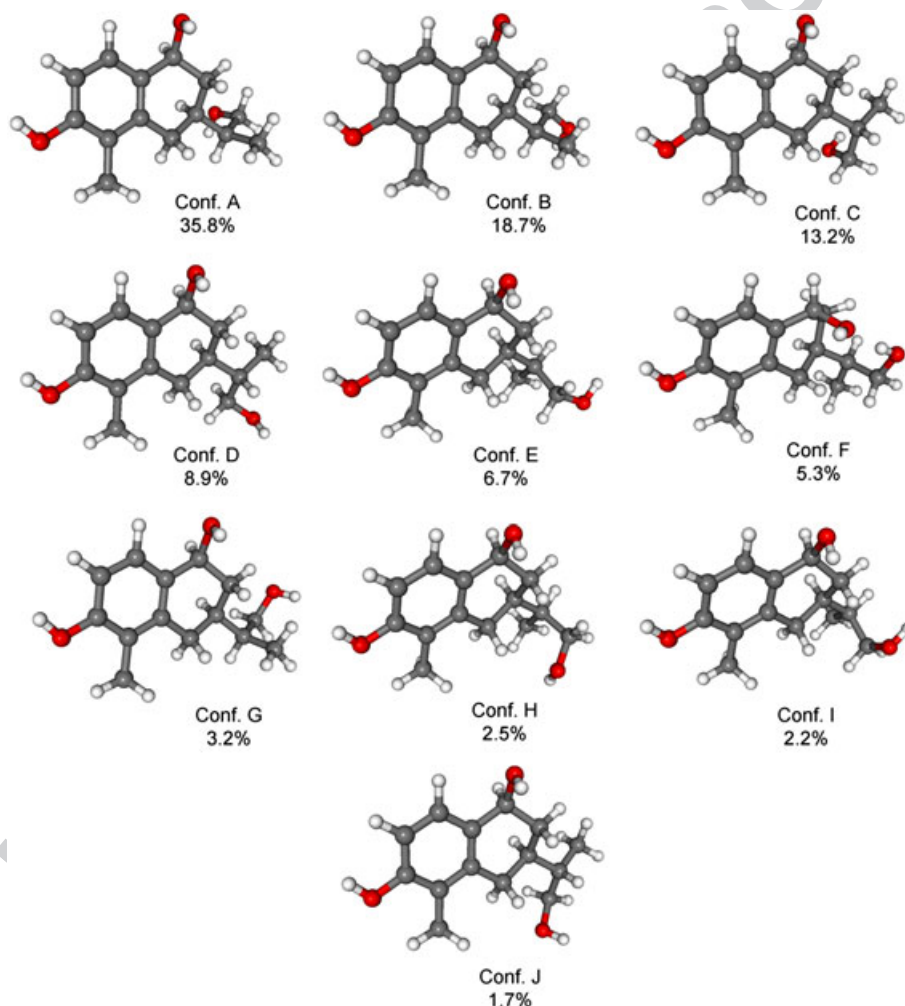


Fig. 7. Low-energy conformers (>1%) of *cis*-(1S,3S,9S)-**1** optimized at B3LYP/TZP level of theory with PCM model for MeCN.

should follow the same inverse helicity rule as chiral 6-hydroxy tetralins. Thus, the measured positive 1L_b band CE derives from *M* helicity of the fused carbocyclic ring and *trans* relative configuration of the equatorial C-3 substituent and the axial 1-OH implies (1S,3*R*) absolute configuration. The *Chirality* DOI 10.1002/chir

remote C-9 chirality center has negligible influence on the ECD spectrum and hence it does not disturb the helicity rule.

In order to confirm the application of the inverse tetralin helicity rule for the configurational assignment of **1**, TDDFT ECD calculations were carried out on the computed solution

conformers of *trans*-(1*S*,3*R*,9*S*)-**1**. Above 2% population, nine conformers of *trans*-(1*S*,3*R*,9*S*)-**1** were identified after reoptimization at the B3LYP/TZVP level of theory with PCM solvent model for F5 acetonitrile (Fig. 5). In all the conformers, the fused carbocyclic ring had *M* helicity ($\omega_{C-8a,C-1,C-2,C-3} = -48.4^\circ$ for the lowest-energy conformers) with axial 1-OH and equatorial C-3 substituent. The conformers differed in the orientation of the C-3 substituent. Then ECD spectra of the conformers were calculated using various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set, which consistently reproduced the experimental solution ECD spectrum including the characteristic positive 1L_b F6 band (Fig. 6). Thus, the (+)-(1*S*,3*R*) absolute configuration of **1** and the effect of the C-6 hydroxyl group on the 1L_b band CE of **1** was unambiguously confirmed.

Since solution conformers of *cis*-(1*S*,3*S*,9*S*)-**1** had been already computed to aid the assignment of the relative configuration, their TDDFT-ECD calculations were also performed (Fig. 5). Above 1.0% population, 10 conformers were obtained, in all of which the C-3 substituent was equatorial and except for conformer F (5.3%), the 1-OH also adopted the equatorial position (Fig. 7). Thus, except for conformer F, the fused carbocyclic ring of the conformers had *P* helicity with $\omega_{C-8a,C-1,C-2,C-3} = 49.9^\circ$ for the lowest-energy conformer (35.8%). In accordance with the inverse tetraline helicity rule, the Boltzmann-weighted TDDFT-ECD spectra of *cis*-(1*S*,3*S*,9*S*)-**1** produced a negative 1L_b band CE, opposite to that of the experimental one, while positive CEs were computed in the 250–200 nm range (1L_a and 1B) but their relative intensities were different from the experimental ones.

CONCLUSIONS

The TDDFT-ECD calculations of (+)-lugikangtinol (**1**) confirmed that the sign of the 1L_b band CE is characteristic of the helicity and hence the absolute configuration of the fused nonaromatic ring and the semiempirical helicity rules of chiral substituted tetralins are useful in determining the absolute configuration when the effect of the aromatic substitution pattern on the sign of the 1L_b band CE is properly considered. The substituted chiral tetralin chromophore is found in pharmacologically active derivatives, the absolute configuration of which could have been readily determined by means of the tetralin helicity rules.^{19–24}

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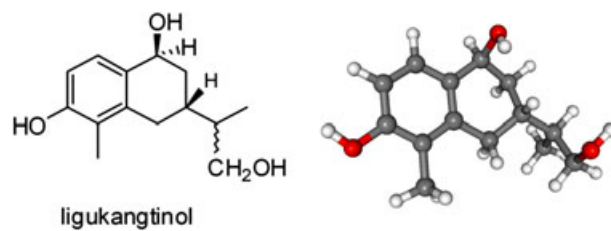
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Regular Article

Structural and Stereochemical Studies of a Tetralin Norsesquiterpenoid from *Ligularia kangtingensis*

Guangbo Xie, Jin Tian, Katalin E. Kövér, Attila Mándi and Tibor Kurtán



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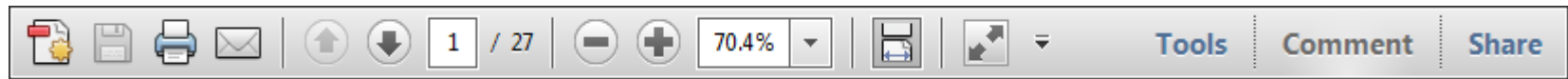
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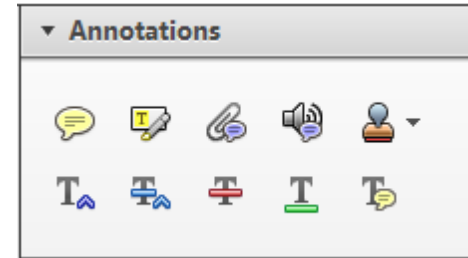
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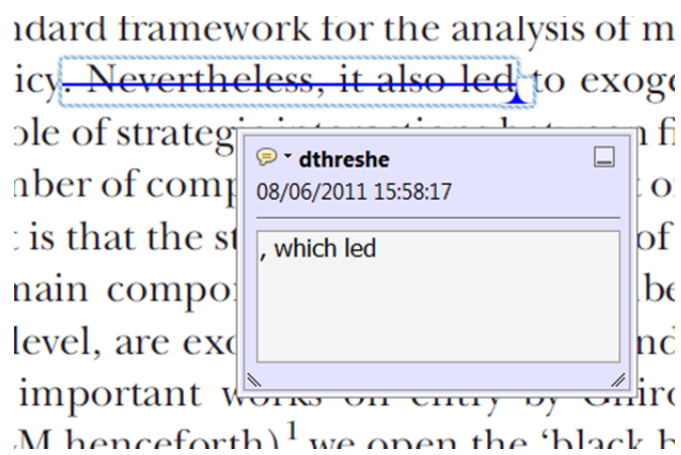
1. Replace (Ins) Tool – for replacing text.



Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.



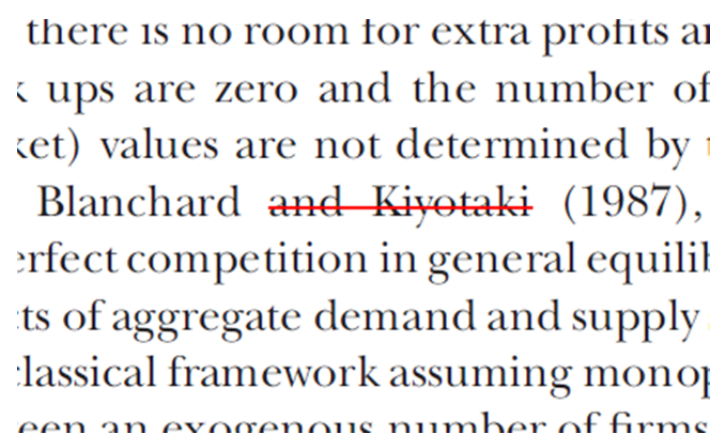
2. Strikethrough (Del) Tool – for deleting text.



Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.



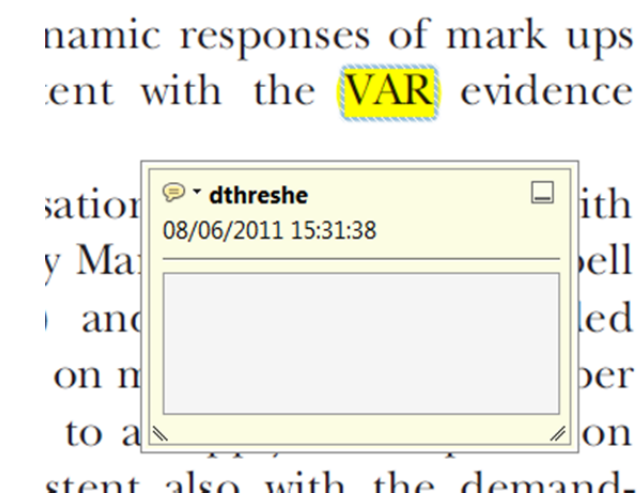
3. Add note to text Tool – for highlighting a section to be changed to bold or italic.



Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.



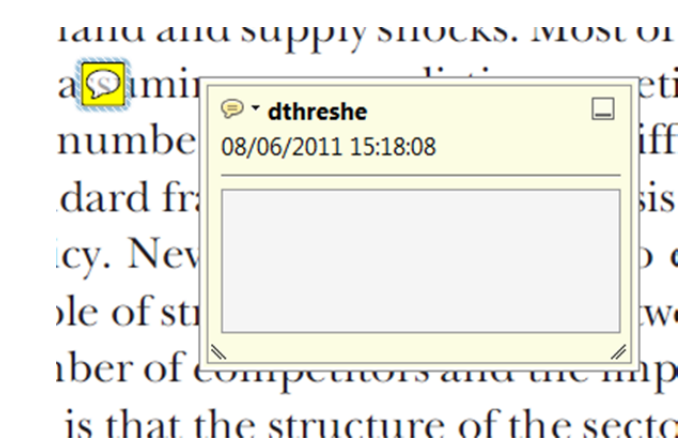
4. Add sticky note Tool – for making notes at specific points in the text.



Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the [Add sticky note](#) icon in the Annotations section.
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- Type the comment into the yellow box that appears.



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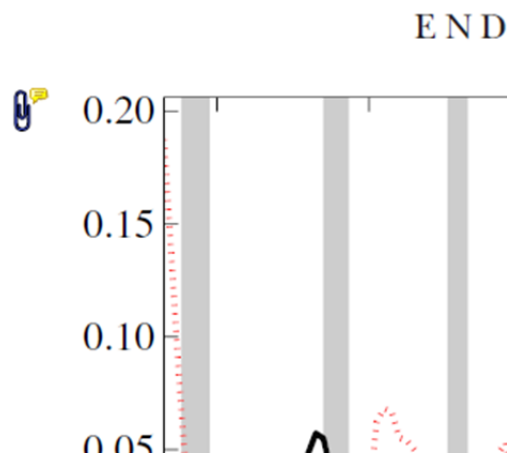
5. Attach File Tool – for inserting large amounts of text or replacement figures.



Inserts an icon linking to the attached file in the appropriate place in the text.

How to use it

- Click on the [Attach File](#) icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
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6. Add stamp Tool – for approving a proof if no corrections are required.

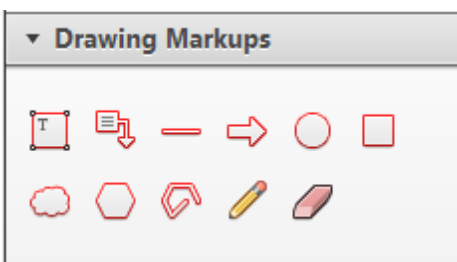


Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the [Add stamp](#) icon in the Annotations section.
- Select the stamp you want to use. (The [Approved](#) stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

of the business cycle, starting with the
 on perfect competition, constant return
 production. In this environment goods
 extra profits and the number of firms
 he number of firms is determined by
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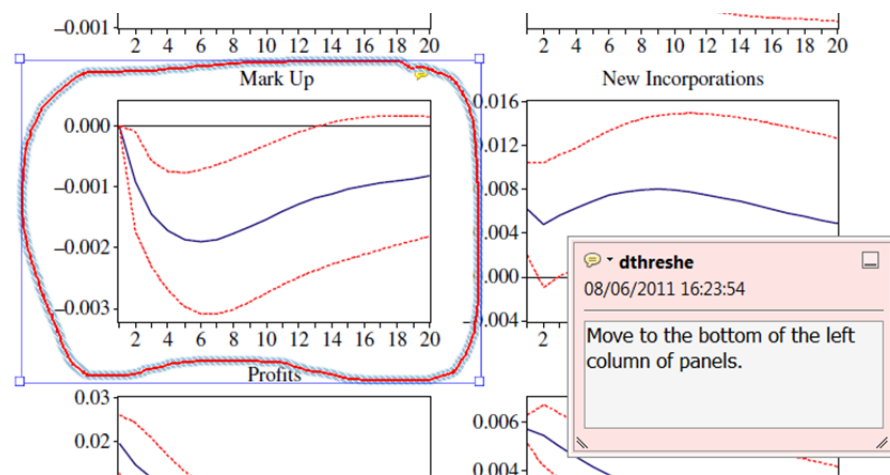


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

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- Double click on the shape and type any text in the red box that appears.



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