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LC–UV-Guided Isolation and Structure Determination of Lancolide E: A Nortriterpenoid with a Tetracyclo[5.4.0.0^{2,4}.0^{3,7}]undecane-Bridged System from a "Talented" *Schisandra* Plant

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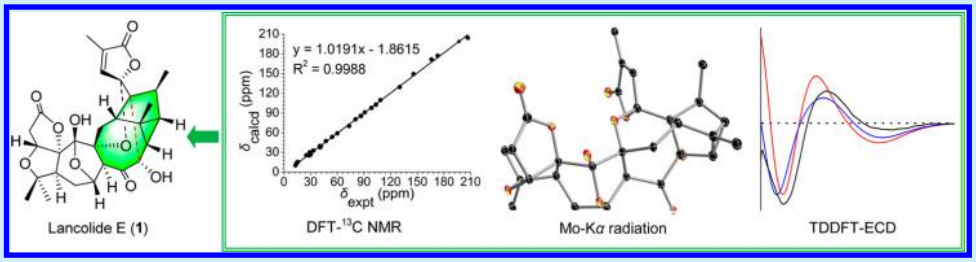
Supporting Information



ABSTRACT: Lancolide E (**1**) featuring a complex tetracyclo[5.4.0.0^{2,4}.0^{3,7}]undecane-bridged system that is constructed by an eight-, a three-, and two five-membered carbon rings in a sterically congested region was obtained in trace amounts from a "talented" schinortriterpenoid producer *Schisandra lancifolia*. Its structure was fully characterized by combining 2D NMR spectroscopy, theoretical calculations, and X-ray diffraction analysis. The biogenetic pathway of **1** was proposed to involve a Prins cyclization.

Since the discovery of the first schinortriterpenoid (SNT) from *Schisandra micrantha* in 2003,[1] plants of the Schisandraceae family, an important medicinal taxon, have become famous for their ability to produce highly oxygenated, rearranged nortriterpenoids. Their underlying biogenetic pathways are thought to originate from 3,4:9,10-disecocycloartanes, which show intriguing terpenoid oxidation and cyclization reactions occurring in nature.[2] Such complex chemical reactions generate tremendous structural variety that the skeletal repertoire is far more diverse than initially thought. In this context, enormous efforts directed toward the total synthesis of these challenging molecules have been ongoing for more than 10 years in several research groups,[2,3] and recently, the complete syntheses of schindilactone A,[3a] rubriflordilactone A,[3c,h] schilancitrilactones B and C,[3f] and propindilactone G[3g] have been accomplished successively, providing a cornucopia of reports on synthetic strategies and tactics.

In our search for new SNTs from plants of the Schisandraceae family, we attempted to identify new architectures in a structure-guided isolation approach. HPLC–UV screening enables simple, rapid, and direct identification of the structural features of SNTs, which is based on our long-established SNTs library. By using this approach, we recently discovered several SNTs bearing unusual scaffolds from *S. lancifolia* collected in the Nujiang prefecture such as schilancitrilactones A–C,[4] lancolides A–D,[5] and lancifonins E and F.[6] Meanwhile, inspecting the isolates and the data from HPLC analysis, we noticed that almost all SNTs from this species showed a maximum UV absorption band in the range of 270–300 nm with slight differences in peak shapes, which is an attribute of the $\alpha,\beta,\gamma,\delta$-unsaturated-$\gamma$-lactone moiety in the side chain or exocyclic position.[2b] Therefore, if there is an exception to the aforementioned cases in *S. lancifolia*, it allows us to assume that the structural feature may have changed and thus a new compound appears to be prospective. As a part of our ongoing research for structurally fascinating SNTs from such a "talented" producer using UV screening approach, a minor SNT (1.3 mg) with a λ_{max} at 204 nm featuring a complex tetracyclo[5.4.0.0^{2,4}.0^{3,7}]undecane-bridged system, termed lancolide E (**1**), was isolated (Figure 1). Herein, we report the isolation, identification by using 2D NMR spectroscopy, theoretical calculations, X-ray diffraction analysis, and biogenetic pathway of **1**.

Lancolide E (**1**) was obtained as white amorphous powder. Its molecular formula was determined as $C_{29}H_{32}O_{10}$ by the positive ESIMS (*m*/*z* 563 $[M + Na]^+$) and HREIMS (*m*/*z* 540.1998 $[M]^+$, calculated 540.1995), indicating 14 degrees of unsaturation. The ^{1}H NMR spectrum of **1** (Table S1, Supporting Information) recorded in acetone-d_6 showed one secondary

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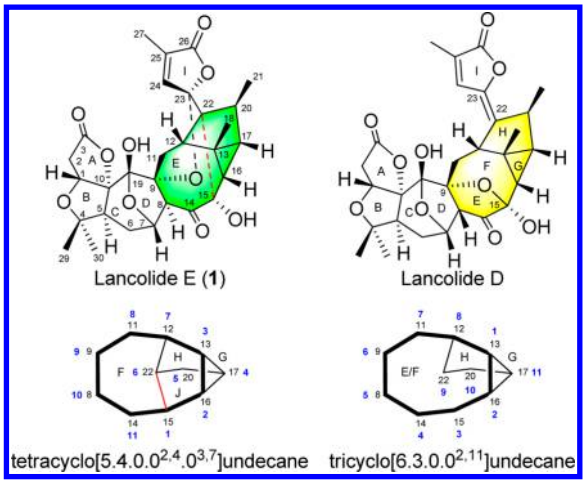

Figure 1. Structures of lancolides D and E (**1**) and their respective nomenclatures of the unique bridged systems (numbering in blue).

methyl at δ_H 1.04 (d, J = 7.0 Hz) and four tertiary methyls at δ_H 1.19 (s), 1.25 (s), 1.26 (s), and 1.96 (br s). An olefinic proton resonance at δ_H 7.28 (d, J = 1.6 Hz) suggested the existence of a trisubstituted double bond. In the lower-field region, four resonances at δ_H 4.23 (br s), 4.39 (s), 4.83 (dd, J = 5.1, 2.5 Hz), 4.97 (d, J = 6.0 Hz), and 6.25 (s) were ascribed to either methines or exchangeable hydroxyl groups. Its ^{13}C NMR and DEPT spectra (Table 1) exhibited 29 carbon resonances, including five methyls, three methylenes, nine methines (one olefinic and two oxygenated carbons), and 12 quaternary carbons (one keto carbonyl, two ester groups, one olefinic carbon, and six oxygenated carbons). By analysis of the HSQC spectrum, all proton resonances were unambiguously assigned to their respective carbons except for the two singlets at δ_H 4.39 (s) and 6.25 (s), which indicated that these two proton resonances were from hydroxyl groups. These observations occupied 4 out of 14 degrees of unsaturation, indicative of an SNT with a decacyclic structure for **1**.

Extensive analysis of the 1D (Table 1) and 2D (Figure 2) NMR data demonstrated that **1** possessing rings A–D and G shared partially structural similarities with lancolide D, a 12,22-cyclopreschisanartane-type SNT that was isolated from this species previously,[5] but the analysis also revealed some significant functional group and structural distinctions between them. The resonances of the C-22/C-23/C-24/C25 conjugated double bond (δ_C 139.6, 141.4, 135.9, and 127.0) and the characteristic C-15 hemiketal carbon resonance (δ_C 106.7) in lancolide D were absent. Instead, a trisubstituted double bond (δ_C 149.3 and 129.4) and three anomalous quaternary carbon resonances (δ_C 109.6, 91.1, and 53.6) were observe in **1**. Additionally, the UV spectrum of lancolide D showed a λ_{max} at 304 nm that was contributed by the exocyclic $\alpha,\beta,\gamma,\delta$-unsaturated γ-lactone moiety,[5] while the λ_{max} of **1** was blue-shifted to 204 nm indicating a rupture in the $\alpha,\beta,\gamma,\delta$-unsaturated γ-lactone but yet suggestive of an α,β-unsaturated-γ-lactone moiety.[7]

The aforementioned differences allowed us to postulate that the tricyclo[6.3.0.0^{2,11}]undecane-bridged system involving the exocyclic conjugated double bond in lancolide D is rearranged in **1**. This assumption was confirmed by detailed analysis of HMBC and ^{1}H–^{1}H COSY data recorded in acetone-d_6 as well as in pyridine-d_5 (Figure 2). The hemiketal C-15 resonance (δ_C 106.7) in lancolide D had been replaced by an oxygenated quaternary carbon resonance at δ_C 91.1 in the eight-membered

Table 1. Experimental and Calculated ^{13}C (150 MHz) NMR Data of Lancolide E (1) (δ in ppm)

	expt		calcd	
no.	δ_C, type[a]	δ_C, type[b]	δ_C[c]	$\Delta\delta_C$[d]
1	80.5, CH	80.8, CH	80.6	0.1
2	39.8, CH_2	40.4, CH_2	40.3	0.5
3	177.2, C	178.1, C	173.0	4.2
4	85.7, C	85.8, C	86.5	0.8
5	47.4, CH	47.4, CH	47.2	0.2
6	33.0, CH_2	32.8, CH_2	33.2	0.2
7	70.2, CH	70.2, CH	73.3	3.1
8	59.0, CH	59.9, CH	60.0	1.0
9	85.3, C	85.6, C	86.8	1.5
10	97.8, C	98.5, C	98.4	0.6
11	25.5, CH_2	26.4, CH_2	28.6	3.1
12	37.6, CH	37.8, CH	40.7	3.1
13	27.2, C	27.3, C	30.3	3.1
14	204.3, C	206.3, C	206.9	2.6
15	91.1, C	91.2, C	91.0	0.1
16	27.0, CH	27.6, CH	29.2	2.2
17	28.5, CH	28.5, CH	29.3	0.8
18	13.9, CH_3	14.0, CH_3	14.4	0.5
19	102.7, C	103.0, C	103.5	0.8
20	39.4, CH	39.5, CH	41.6	2.2
21	15.6, CH_3	16.0, CH_3	15.7	0.1
22	53.6, C	53.8, C	54.3	0.7
23	109.6, C	109.8, C	108.5	1.1
24	149.3, CH	149.6, CH	145.8	3.5
25	129.4, C	129.6, C	130.3	0.9
26	171.5, C	172.5, C	166.1	5.4
27	10.3, CH_3	10.3, CH_3	13.0	2.7
29	25.8, CH_3	26.0, CH_3	25.2	0.6
30	30.4, CH_3	30.7, CH_3	29.1	1.3

[a]Recorded in acetone-d_6. [b]Recorded in pyridine-d_5. [c]Calculated in acetone-d_6. [d]$\Delta\delta_C = |\delta_{calcd} - \delta_{expt}|$, MAE = 1.6 ppm, and CMAE = 2.2 ppm.

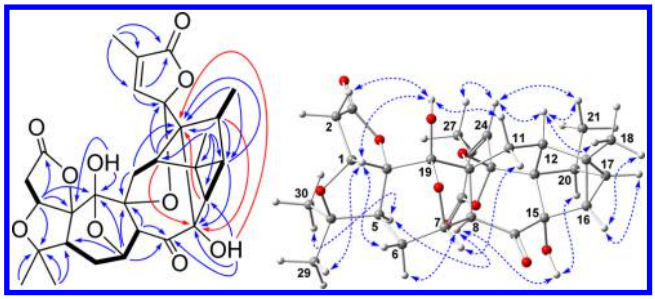

Figure 2. Key HMBC (H → C), ^{1}H–^{1}H COSY(−), and ROESY correlations of **1**.

carbon ring (ring F) of **1**, which was judged by the HMBC correlations (recorded in acetone-d_6) from the hydroxyl group resonance at δ_H 4.39 (HO-15) to C-14 (δ_C 204.3), C-15 (δ_C 91.1), and C-16 (δ_C 27.0) together with the correlations (recorded in pyridine-d_5) from H-16 (δ_H 1.97, d, J = 5.5 Hz) and H-17 (δ_H 1.18, d, J = 5.5 Hz) to C-15 (δ_C 91.2). Although the chemical shift of C-9 in **1** was almost identical with that in lancolide D, the disappearance of a hemiketal resonance in ring F of **1** indicated a cleavage of the oxa bridge formed between C-9 and C-15. The quaternary carbon resonance at δ_C 53.6 was assigned to C-22 in ring H because it showed HMBC correlations (recorded in acetone-d_6) with H-11α (δ_H = 2.48, ddd,

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J = 14.6, 11.1, 1.0 Hz), H-12 (δ_H = 2.31, dd, J = 11.1, 6.2 Hz), H-20 (δ_H = 2.67, m), and Me-21 (δ_H = 1.04, d, J = 7.0 Hz). Most notably, HMBC correlations (recorded in acetone-d_6) from HO-15 and H-16 to C-22 and from H-12 to C-15 together with correlation (recorded in pyridine-d_5) from H-20 to C-15, which were not presented in lancolide D, were observed in **1**, and thus, this set of HMBC correlations required that there was a carbon−carbon linkage between C-15 and C-22, which formed a five-membered carbon ring (ring J) containing C-15, C-16, C-17, C-20, and C-22. Therefore, a complex, sterically congested tetracyclo[5.4.0.0^{2,4}.0^{3,7}]undecane-bridged system constructed by an eight-, a three-, and two five-membered carbon rings was established. Subsequently, analysis of the HMBC correlations (recorded in acetone-d_6) from a tertiary methyl (δ_H 1.96) to two olefinic carbon resonances (δ_C 149.3 and 129.4) and an ester group (δ_C 171.5) and from an olefinic proton resonance (δ_H = 7.28, d, J = 1.6 Hz) to the ester group and a quaternary carbon resonance (δ_C 109.6) demonstrated the existence of an α,β-unsaturated γ-lactone moiety (ring I) containing C-23, C-24, C-25, and C-26. In addition, linkage of rings H and I by a carbon−carbon single bond could be deduced from the HMBC correlation (recorded in acetone-d_6) from H-12 to C-23. Considering the molecular formula and the remaining one degree of unsaturation unaccounted for, C-9 and C-23 should be attached to the oxygen atom left to form a six-membered oxygen-containing ring (ring E) that was connected to ring I in a spirocyclic manner at C-23, thus completing the planar structure of **1** (Figure 1).

The rigidity of **1** facilitated the assignment of the relative configuration. The relative configuration of chiral centers in rings A−D was determined to be the same as those in lancolide D by the similar ROESY correlations (Figure 2) and carbon (Table 1) and proton (Table S1, Supporting Information) chemical shifts of both compounds, except that H-8 in **1** was assigned to be α-orientated via the ROESY correlations of H-8 (δ_H = 4.23, br s) with H-5 (δ_H = 2.64, m) and H-6α (δ_H = 1.94, m). The observation of ROESY correlations of HO-15 with H-8 and H-20 indicated that HO-15 and H-20 were α-orientated. Moreover, the ROESY correlations of H-12 with Me-18 and Me-21; and of H-16 (δ_H = 1.59, d, J = 5.4 Hz) with H-17 (δ_H = 1.20, overlap) and Me-18 were observed as well, thus leading to assignments of a β-orientation to H-12, H-16, H-17, Me-18, and Me-21. The R* configuration for C-23 in the spiro lactone was determined by the ROESY correlations of H-24 with H-12 and Me-21. Thus, the relative configuration of **1** was determined as 1R*, 5S*,7S*,8S*,9R*,10R*,12S*,13S*,15S*,16S*,17R*,19S*,20S*, 22S*,23R*.

A computational method was carried out to confirm the planar structure and relative configuration of **1** through comparison of its experimental and calculated ^{13}C NMR data due to the initially unsuccessful attempts to obtain single crystals of **1**. The MMFF conformational search resulted in seven conformers in a 30 kJ/mol energy window. The conformational geometries optimization of these conformers at the B3LYP/6-31G(d) level in vacuo afforded one conformer accounting for more than 99% (Table S3, Supporting Information), suggesting that compound **1** presented as a structurally rigid polycyclic construct. In regard to calculation of ^{13}C chemical shifts, nuclear shielding constants were calculated using the GIAO method[8] at the MPW1PW91-SCRF/6-31G(d,p) level in acetone with PCM, and the shieldings so obtained were converted into chemical shifts by referencing to TMS at 0 ppm ($\delta_{calcd} = \sigma_{TMS} - \sigma_{calcd}$). The correlation coefficient (R^2) between calculated and experimental data obtained by linear regression analysis was 0.9988 (Figure 3), and the mean absolute error (MAE) and the corrected mean absolute error (CMAE) were 1.6 and 2.2 ppm (Table 1), respectively, thus supporting the structure furnished by 1D and 2D NMR data.

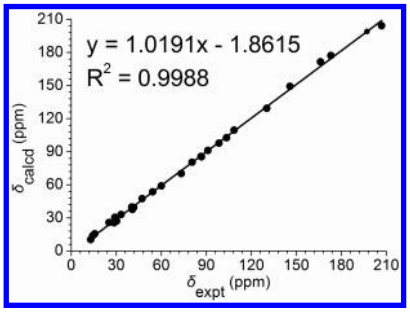

Figure 3. Regression analysis of experimental versus calculated ^{13}C NMR chemical shifts of **1**; linear fitting was shown as a line.

Various recipes and solvent systems were employed to address the issue of growing suitable single crystals for X-ray diffraction experiment so as to provide solid evidence of the structure of **1**. Slow evaporation, slow cooling, and solvent diffusion methods with single and binary solvent systems were first used. However, the evaporation rate was carefully controlled but amorphous powders rather than crystals were obtained. The vapor diffusion method was then tried, yet slow diffusion of poor solvents, i.e., chloroform, petroleum ether, 2-propanol, and ethyl acetate, into the clear solutions of methanol, acetone, or pyridine containing **1**, respectively, led to the formation of noncrystalline powders as well. Fortunately, when water was used as a poor solvent to slowly diffuse into a solution of methanol containing **1** at room temperature, suitable crystals of **1** in the form of colorless prisms were ultimately obtained. The X-ray diffraction analysis using Mo Kα radiation revealed that **1** crystallized in the monoclinic chiral P21 space group and its asymmetric unit contained two crystallographically independent molecules with same configurations (Figure S1, Supporting Information). Further investigation indicated that **1** exhibited twinning, and therefore, the structure was refined employing the TWIN and BASF instructions in SHELXL-97,[9] generating the final BASF value of 0.217. Thus, the structure and relative configuration of **1** were successfully established (CCDC 1427792, Figure 4).

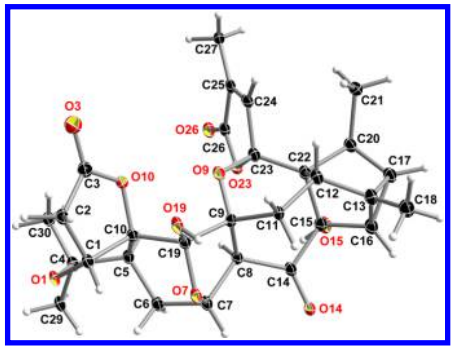

Figure 4. ORTEP representation of crystal structure of **1**.

The absolute configuration of **1** was assigned as 1R,5S,7S,8S, 9R,10R,12S,13S,15S,16S,17R,19S,20S,22S,23R based on the conservation of the absolute stereochemistries of the western

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hemisphere of SNTs. Subsequently, time-dependent density-functional theory (TDDFT) method[10] at the CAM-B3LYP/TZVP level in the gas phase and in methanol with PCM was employed to simulate the electronic circular dichroism (ECD) spectra of **1**. Close agreement was found in the calculated and experimental curves (Figure 5). Molecular orbital (MO) analysis (Figure S3, Supporting Information) at the same level in methanol revealed that the weak Cotton effect (CE) in the experimental curve at 301 nm could be ascribed to the negative rotatory strength at 288.6 nm that was generated by the electronic transitions from MO143 to MO145 in the cyclopropyl moiety and the carbonyl group; the experimental positive CE at 248 nm could be assigned to the positive rotatory strength at 235.6 nm that was contributed by the electronic transitions from MO137 to MO144; the diagnostic negative CE at 213 nm could be ascribed to the negative rotatory strength at 209.6 nm from MO139 to MO144 involving a $\pi \rightarrow \pi^*$ transition of in the α,β-unsaturated-γ-lactone moiety. Therefore, the absolute configuration of **1** was defined to be 1*R*,5*S*,7*S*,8*S*,9*R*,10*R*,12*S*,13*S*,15*S*,16*S*,17*R*,19*S*,20*S*,22*S*,23*R*.

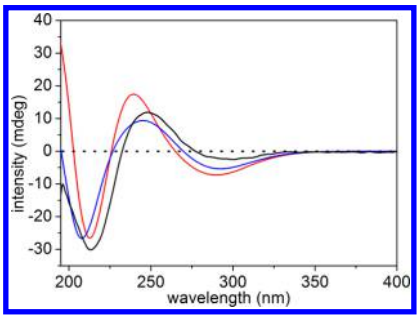
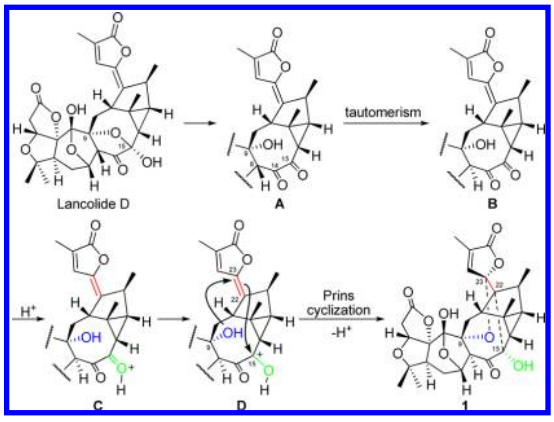


Figure 5. Experimental and calculated ECD spectra of **1** (black, experimentally observed in methanol; blue, calculated in the gas phase; red, calculated in methanol).

In the structural elucidation of lancolide E (**1**), we became aware of a biogenetic relationship between lancolides D and E (**1**) (Scheme 1). It is likely that a cleavage of the hemiketal in lancolide D affords intermediate A that forms intermediate B, a C-8 epimer, via a keto−enol tautomerism. A subsequent Prins cyclization catalyzed by organic acids[11] of intermediate B is as a key step to constructing the C-15/C-22 carbon bond and the oxa bridge between C-9 and C-23. Due to sample quantity limitations, bioactivity evaluation of **1** was not feasible.

Scheme 1. Proposed Biogenetic Pathway for 1



■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03334.

Detailed experimental procedures, physical−chemical properties, 1D and 2D NMR, MS, UV, and ECD spectra, and computational data of compound **1** (PDF)

X-ray data of **1** (CIF)

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Notes

The authors declare no competing financial interest.

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