Stereochemical Revision of Xylogranatin F by GIAO and DU8+ NMR Calculations

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Keywords: DU8+, GIAO NMR calculation, limonoid, structural reassignment, natural product

Abstract: This manuscript describes predicted NMR shifts for the limonoid natural product xylogranatin F. The ^{1}H and ^{13}C NMR shifts of four diastereomers were evaluated by more conventional GIAO and more modern DU8+ methods. The results of the ^{1}H and ^{13}C NMR calculations for both the GIAO method and DU8+ calculations suggest the structure which was recently reassigned by chemical synthesis. Furthermore, we show that DU8+ provides superior results with less computation time.

Introduction

NMR calculations have increasingly become a useful and widely employed tool in both structural determination and revision of complicated molecules, especially in the context of natural products isolation and multistep synthesis.[1-2] Utilization of modern quantum chemical computations has the advantage of magnifying and quantifying subtle differences among similar structures (e.g. diastereomers).[3] Using a computational approach for structure determination instead of a purely spectroscopic one has the advantage of only requiring minimal quantities of material to obtain basic spectroscopic data unlike other methods, most notably 2D NMR techniques. Furthermore, a computationally powered approach has the advantage over authentic synthesis.

During the course of our total synthesis of granatumine A and related bislactone limonoid alkaloids (**1**-**2**), we determined by chemical synthesis that xylogranatin F (**3**) was structurally mischaracterized (Figure 1).[4] Herein we report the full analysis of the NMR shift calculations of the four most likely diastereomers by both GIAO and DU8+, which provide an approach for conducting NMR calculations within this class to provide results consistent with authentic synthesis.

In the original isolation paper, the authors proposed that xylogranatin F had the structure **3** wherein the relative configuration at positions C3, C5, and C10 was defined through two key NOESY contacts (Figure 1).[5] Although the NOE signal between the protons at C5 and C19 (the substituent at C10) is unequivocal and establishes the *cis* fusion between rings A and F, the asserted NOE signals between the protons at C3 and C5, as well as between C3 and C19 were weak and ambiguous. It was unclear if what was interpreted as a signal was in fact an authentic NOE, or if instead it could be attributed to background noise. Suspecting a structural misassignment, we attempted to resolve this issue based on NMR calculations.

We first considered four different possible structures (**3**-**6**) by varying the stereochemistry at positions C3, C5 and C10 while maintaining the *cis*-fusion between ring A and F. The *cis*-fusion is supported by reliable NOESY data, and furthermore by the presumed biosynthesis that involves a lactonization to form the left-most F-ring γ-lactone. Biosynthetically, the ring F lactone is proposed to form via an intramolecular cyclization of the tethered

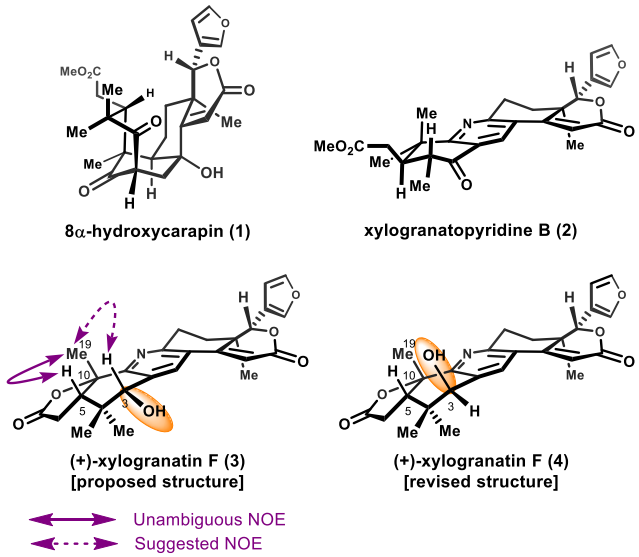

FIGURE 1. Xylogranatin F and Related Limonoid Natural

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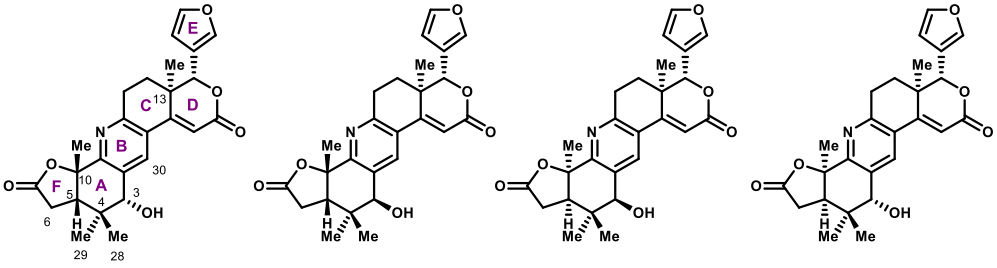
TABLE 1. ^{13}C NMR Chemical Shifts Calculated with GIAO[a]



3*R*-5*S*-10*S* (3) [proposed Structure] | **3*S*-5*S*-10*S* (4) [revised structure]** | **3*S*-5*R*-10*R* (5)** | **3*R*-5*R*-10*R* (6)**

Position	Wu et al. ^{13}C δ[ppm]	**3** δ[ppm]	Δ(**3**-reported) [ppm]	**4** δ[ppm]	Δ(**4**-reported) [ppm]	**5** δ[ppm]	Δ(**5**-reported) [ppm]	**6** δ[ppm]	Δ(**6**-reported) [ppm]
1	156.7	155.4	-1.3	157.0	0.3	155.4	-1.3	156.9	0.2
2	130.6	130.4	-0.2	130.0	-0.6	130.5	-0.1	130.2	-0.4
3	75.6	75.5	-0.1	76.2	0.6	75.6	0.0	76.0	0.4
4	36.3	39.9	3.6	39.2	2.9	40.0	3.7	39.2	2.9
5	45.8	49.8	4.0	46.1	0.3	49.8	4.0	46.2	0.4
6	31.0	33.4	2.4	32.5	1.5	33.4	2.4	32.5	1.5
7	175.2	176.0	0.8	176.4	1.2	176.1	0.9	176.4	1.2
8	124.3	123.5	-0.8	123.4	-0.9	123.5	-0.8	123.4	-0.9
9	158.1	157.4	-0.7	158.4	0.3	157.2	-0.9	158.2	0.1
10	84.1	83.7	-0.4	84.2	0.1	83.4	-0.7	84.0	-0.1
11	28.0	29.8	1.8	29.9	1.9	29.7	1.7	29.9	1.9
12	30.3	31.3	1.0	31.2	0.9	30.9	0.6	31.1	0.8
13	37.7	41.1	3.4	41.1	3.4	41.9	4.2	41.5	3.8
14	157.2	159.8	2.6	159.7	2.5	160.0	2.8	159.7	2.5
15	111.2	110.5	-0.7	110.4	-0.8	110.4	-0.8	110.3	-0.9
16	165.2	164.3	-0.9	164.3	-0.9	164.2	-1.0	164.2	-1.0
17	80.9	79.6	-1.3	79.6	-1.3	81.0	0.1	80.2	-0.7
18	15.8	15.3	-0.5	15.2	-0.6	14.7	-1.1	15.0	-0.8
19	28.5	27.3	-1.2	26.2	-2.3	27.0	-1.5	25.9	-2.6
20	119.8	121.3	1.5	121.1	1.3	121.9	2.1	121.4	1.6
21	141.4	140.8	-0.6	140.8	-0.6	141.2	-0.2	141.0	-0.4
22	110.0	110.2	0.2	110.2	0.2	109.6	-0.4	109.9	-0.1
23	143.3	142.0	-1.3	142.0	-1.3	141.2	-2.1	141.6	-1.7
28	23.5	24.1	0.6	22.3	-1.2	15.4	-8.1	19.2	-4.3
29	21.4	15.6	-5.8	19.2	-2.2	24.0	2.6	22.3	0.9
30	133.9	131.7	-2.2	133.0	-0.9	131.6	-2.3	133.1	-0.8
MAE			1.5		1.2		1.8		1.3
RMSD			2.0		1.5		2.5		1.7
Max			5.8		3.4		8.1		4.3
DP4		0.6%		81.8%		0.0%		17.6%	

[a] Chemical shifts deviation greater than 3.0 ppm are colored in red.

ester, which one might expect would proceed to provide the more stable *cis*-fused relationship.[6] Presumed biosynthesis aside, the spectral data was unambiguous on this matter.

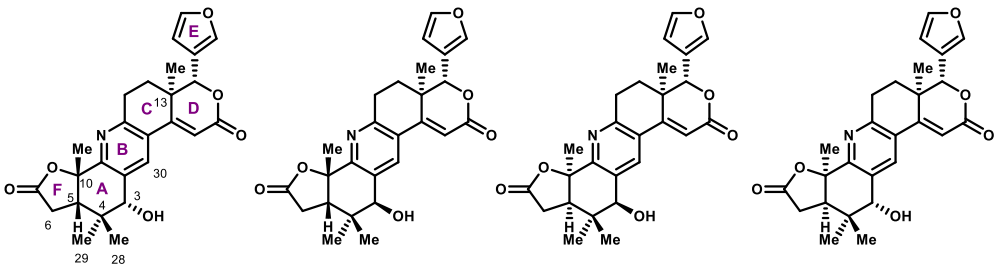
We next conducted NMR calculations of the four diastereomers we considered to be the most likely candidates. In addition to inversion of the originally assigned C3 position – the one position for which biosynthetic considerations did not provide much insight – the pair of stereocenters at C5 and C10 were additionally inverted, maintaining the *cis*-relationship. These additional modifications were evaluated because of the possibility for the inversion of C5 and C10 over the course of the biosynthesis – this being challenging to evaluate given the biosynthesis has not been characterized for the pyridine limonoids.

Materials and Methods

The general workflow for GIAO NMR calculations was performed according to the procedure by Hoye and co-workers.[7-8] 6–12 conformers were generated for each possible structure via molecular mechanics calculations using MacroModel before they were subjected to geometry optimization calculations using Gaussian '09. Geometry optimization calculations were performed in the gas phase using the B3LYP/6-31+G(d,p) level of theory. NMR single point calculations (GIAO) were performed using Gaussian '09 on all geometrically optimized structures using mPW1PW91/6-311+G(2d,p) in chloroform with the SMD solvation method. The NMR and free energy data were assembled and Boltzmann-averaged. All chemical shifts were scaled.[9] The NMR data, as well as maximum and average deviations between the scaled calculated and experimental chemical shifts, are shown in Tables 1–2. The scaled shifts were also subjected to statistical analysis using DP4.[10]

DU8+ calculations were performed as previously described,[11] except the GIAO computations were performed with the Gaussian '09 default PCM method (in chloroform). The structures were pre-

TABLE 2. ^{1}H NMR Chemical Shifts Calculated with GIAO[a]



3*R*-5*S*-10*S* (3) [proposed Structure] | **3*S*-5*S*-10*S* (4) [revised structure]** | **3*S*-5*R*-10*R* (5)** | **3*R*-5*R*-10*R* (6)**

Position	Wu et al. ^{1}H δ[ppm]	**3** δ[ppm]	Δ(**3**-reported) [ppm]	**4** δ[ppm]	Δ(**4**-reported) [ppm]	**5** δ[ppm]	Δ(**5**-reported) [ppm]	**6** δ[ppm]	Δ(**6**-reported) [ppm]
1									
2									
3	4.48	4.40	-0.08	4.34	-0.14	4.41	-0.07	4.33	-0.15
4									
5	2.97	2.43	-0.54	2.90	-0.07	2.42	-0.55	2.90	-0.07
6α	2.58	2.91	0.33	2.44	-0.14	2.79	0.21	2.88	0.30
6β	2.98	2.77	-0.21	2.86	-0.12	2.91	-0.07	2.44	-0.54
7									
8									
9									
10									
11α	3.10	2.96	-0.14	2.98	-0.12	2.98	-0.12	2.97	-0.13
11β	3.20	2.98	-0.22	2.99	-0.21	3.05	-0.15	3.06	-0.14
12α	1.87	1.63	-0.24	1.63	-0.24	1.92	0.05	1.76	-0.11
12β	1.75	1.69	-0.06	1.68	-0.07	1.86	0.11	1.77	0.02
13									
14									
15	6.57	6.37	-0.20	6.34	-0.23	6.39	-0.18	6.34	-0.23
16									
17	5.22	5.07	-0.15	5.07	-0.15	5.12	-0.10	5.09	-0.13
18[b]	1.15	1.13	-0.02	1.14	-0.01	0.98	-0.17	1.07	-0.08
19[b]	1.83	1.64	-0.19	1.69	-0.14	1.64	-0.19	1.68	-0.15
20									
21	7.56	7.23	-0.33	7.24	-0.32	7.41	-0.15	7.32	-0.24
22	6.52	6.52	0.00	6.52	0.00	6.22	-0.30	6.39	-0.13
23	7.48	7.27	-0.21	7.27	-0.21	7.26	-0.22	7.28	-0.20
28[b]	1.16	1.00	-0.16	1.12	-0.04	0.80	-0.36	0.68	-0.48
29[b]	0.83	0.80	-0.03	0.66	-0.17	1.00	0.17	1.11	0.28
30	8.05	8.15	0.10	7.83	-0.22	8.16	0.11	7.83	-0.22
MAE			0.18		0.14		0.18		0.20
RMSD			0.22		0.17		0.22		0.24
Max			0.54		0.32		0.55		0.54
DP4		0.0%		100.0%		0.0%		0.0%	

[a] Chemical shifts deviation greater than 0.20 ppm are colored in red. [b] ^{1}H shifts of homotopic protons were averaged.

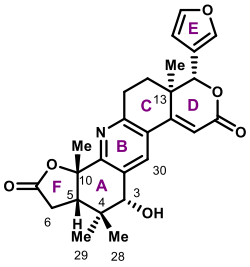
optimized with the force field MMFF94 as implemented in OpenBabel before they were subjected to geometry optimization calculations using Gaussian '09.[12] Geometry optimization calculations were performed using the B3LYP/6-31G(d) level of theory with the default PCM solvation method (chloroform). NMR single point calculations (GIAO) were performed using Gaussian '09 on all geometrically optimized structures using ωB97xD/6-31G(d) in chloroform with the default PCM solvation method. The DU8+ empirical corrections for ^{13}C chemical shifts where then applied.[11] The NMR data were Boltzmann-averaged. The ECD calculations were performed at the B3LYP/6-311+G(d)//B3LYP/6-31+G(d) level of TDDFT theory.

Results and Discussion

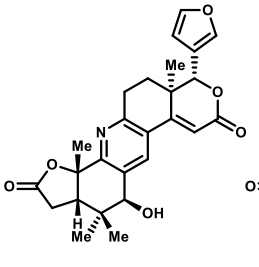
GIAO NMR CALCULATION RESULTS

The results of the GIAO NMR calculations are consistent with the reassignment of xylogranatin F to structure **4**. According to the ^{13}C NMR calculation results, the chemical shifts of the originally proposed structure, 3*R*-5*S*-10*S* (**3**) have an average deviation of 1.5 ppm and RMSD of 2.0 (Table 1). Furthermore, the chemical shifts of several carbons on rings A and F around the C3 stereogenic center differ by more than 3.0 ppm. Specifically, calculated ^{13}C chemical shifts of C4 and C5 on ring A are 3.6 ppm and 4.0 ppm more downfield than the reported values respectively. On the other hand, those of C29 are 5.8 ppm more upfield than the experimentally observed shifts. In comparison, the 3*S*-5*S*-10*S* structure (**4**), i.e. the C3-epimer of the original structure, has the lowest average chemical shifts deviation of 1.2 ppm and RMSD of 1.5. and the deviation between the calculated ^{13}C NMR chemical

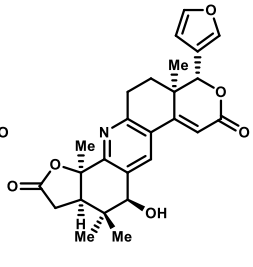
TABLE 3. ^{13}C NMR Chemical Shifts Calculated with DU8+[a]



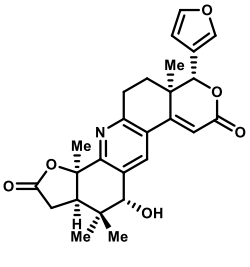
3*R*-5*S*-10*S* (3)
[proposed Structure]



3*S*-5*S*-10*S* (4)
[revised structure]



3*S*-5*R*-10*R* (5)



3*R*-5*R*-10*R* (6)

Position	Wu et al. ^{13}C δ[ppm][5]	**3** δ[ppm]	Δ(**3**-reported) [ppm]	**4** δ[ppm]	Δ(**4**-reported) [ppm]	**5** δ[ppm]	Δ(**5**-reported) [ppm]	**6** δ[ppm]	Δ(**6**-reported) [ppm]
1	156.7	156.0	-0.7	157.7	1.0	155.8	-0.9	157.6	0.9
2	130.6	131.6	1.0	131.9	1.3	131.6	1.0	131.9	1.3
3	75.6	75.6	0.0	76.2	0.6	75.6	0.0	76.2	0.6
4	36.3	37.1	0.8	35.8	-0.5	37.0	0.7	35.7	-0.6
5	45.8	48.8	3.0	46.8	1.0	48.8	3.0	46.6	0.8
6	31.0	31.7	0.7	31.4	0.4	31.8	0.8	31.4	0.4
7	175.2	174.5	-0.7	174.4	-0.8	174.5	-0.7	174.5	-0.7
8	124.3	124.9	0.6	124.8	0.5	125.0	0.7	124.8	0.5
9	158.1	158.3	0.2	159.4	1.3	158.4	0.3	159.5	1.4
10	84.1	85.2	1.1	86.1	2.0	85.0	0.9	85.9	1.8
11	28.0	28.5	0.5	28.6	0.6	28.5	0.5	28.6	0.6
12	30.3	30.3	0.0	30.2	-0.1	30.3	0.0	30.3	0.0
13	37.7	37.8	0.1	37.9	0.2	37.9	0.2	37.8	0.1
14	157.2	157.8	0.6	157.6	0.4	157.7	0.5	157.4	0.2
15	111.2	111.8	0.6	111.7	0.5	111.8	0.6	111.9	0.7
16	165.2	165.2	0.0	165.1	-0.1	165.2	0.0	165.1	-0.1
17	80.9	82.0	1.1	81.9	1.0	81.8	0.9	81.9	1.0
18	15.8	16.1	0.3	16.1	0.3	16.2	0.4	16.1	0.3
19	28.5	28.2	-0.3	28.5	0.0	28.0	-0.5	28.3	-0.2
20	119.8	121.0	1.2	120.8	1.0	120.9	1.1	120.7	0.9
21	141.4	141.4	0.0	141.2	-0.2	141.2	-0.2	141.3	-0.1
22	110.0	109.8	-0.2	109.7	-0.3	109.7	-0.3	109.7	-0.3
23	143.3	142.4	-0.9	142.3	-1.0	142.3	-1.0	142.3	-1.0
28	23.5	24.2	0.7	23.2	-0.3	24.1	0.6	23.2	-0.30
29	21.4	13.8	-7.6	20.9	-0.5	13.7	-7.7	20.7	-0.66
30	133.9	132.6	-1.3	134.0	0.1	132.8	-1.1	133.8	-0.1
MAE			0.9		0.6		0.9		0.6
RMSD			1.7		0.8		1.7		0.7
Max			7.6		2.0		7.7		1.8

[a] Chemical shifts deviation greater than 3.0 ppm are colored in red.

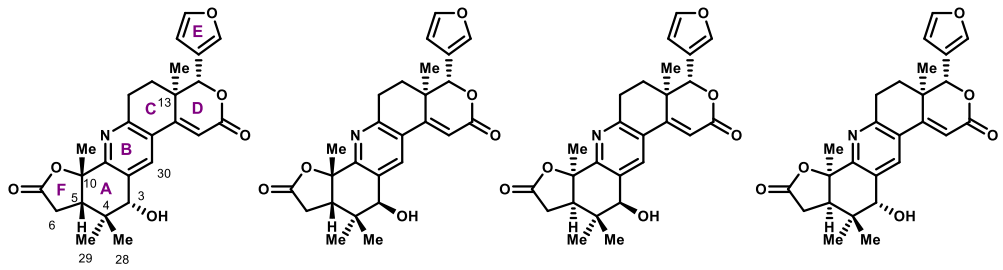
shifts of C4, C5, C29 for **4** and the reported ^{13}C NMR shifts are all within 3.0 ppm. In addition, **4** is predicted to be the correct structure with 81.8% probability by DP4 analysis based on the ^{13}C NMR shifts.[10]

The ^{1}H NMR calculation results also suggest the structure misassignment (Table 2). The chemical shifts of the originally proposed structure, 3*R*-5*S*-10*S* (**3**) have an average deviation of 0.18 ppm and RMSD of 0.22 (Table 1). In addition, the deviation between the calculated ^{1}H NMR chemical shifts of the protons on C5 and C6 and the values reported by Wu et al. are more than 0.20 ppm. In comparison, the 3*S*-5*S*-10*S* structure (**4**) has the lowest average chemical shifts deviation of 0.14 ppm with RMSD of 0.17. and the deviation between the calculated ^{1}H-NMR chemical shifts of the protons on C5 and C6 for **4** and the reported ^{13}C NMR shifts are all within 0.20 ppm. Additionally, **4** is predicted to be the correct structure with 100.0% probability using the ^{1}H-NMR chemical shifts only as well as using both proton and carbon data by DP4 analysis.

DU8+ CALCULATION RESULTS

The results of DU8+ calculations confirmed the misassignment (Tables 3 and 4). The most striking discrepancy between the experimental ^{13}C NMR chemical shift data and data calculated for the originally proposed structure **3** was the chemical shift of Me29 (off by 7.6 ppm). That of C5 also deviated by 3.0 ppm. In contrast, the 3*S*-5*S*-10*S* structure (**4**) gave an excellent agreement with the experimental data: RMSD = 0.8 ppm, MAE = 0.6 ppm, and the maximum deviation of 2.0 ppm. However, the five-bond separation between the closest stereogenic centers C3 and C13 in the two pyridine-separated cyclohexene moieties makes it unlikely to differentiate between diastereomers **4** and **6** based solely on the calculated NMR data. The same is true for the pair of diastereomers **3** and **5**, which are also indistinguishable based on the calculated NMR data. It is understandable that with separation of stereogenic centers, the difference in ^{13}C NMR chemical shifts for the two diastereomers becomes less pronounced such that it is below the accuracy of the computational method. The fact that the calculated ^{13}C NMR shifts for diastereomers **4** and **6** are so similar, points to the obvious

TABLE 4. ^{1}H NMR Chemical Shifts Calculated with DU8+[a]



3*R*-5*S*-10*S* (3) [proposed Structure] | **3*S*-5*S*-10*S* (4) [revised structure]** | **3*S*-5*R*-10*R* (5)** | **3*R*-5*R*-10*R* (6)**

Position	Wu et al. ^{1}H δ[ppm][5]	**3** δ[ppm]	Δ(**3**-reported) [ppm]	**4** δ[ppm]	Δ(**4**-reported) [ppm]	**5** δ[ppm]	Δ(**5**-reported) [ppm]	**6** δ[ppm]	Δ(**6**-reported) [ppm]
1									
2									
3	4.48	4.74	0.26	4.73	0.25	4.77	0.29	4.70	0.22
4									
5	2.97	2.51	-0.46	3.15	0.18	2.51	-0.46	3.15	0.18
6α	2.58	2.71	0.13	2.56	-0.02	2.72	0.14	2.57	-0.01
6β	2.98	3.03	0.05	2.93	-0.05	3.03	0.05	2.95	-0.03
7									
8									
9									
10									
11α	3.10	3.23	0.13	3.23	0.13	3.18	0.08	3.19	0.09
11β	3.20	3.17	-0.03	3.19	-0.01	3.24	0.04	3.24	0.04
12α	1.87	1.83	-0.04	1.84	-0.03	1.82	-0.05	1.80	-0.07
12β	1.75	1.87	0.12	1.86	0.11	1.90	0.15	1.88	0.13
13									
14									
15	6.57	6.41	-0.16	6.34	-0.23	6.46	-0.11	6.38	-0.19
16									
17	5.22	5.31	0.09	5.30	0.08	5.29	0.07	5.29	0.07
18[b]	1.15	1.37	0.22	1.35	0.20	1.37	0.22	1.37	0.22
19[b]	1.83	1.78	-0.05	1.92	0.09	1.77	-0.06	1.91	0.08
20									
21	7.56	7.62	0.06	7.62	0.06	7.61	0.05	7.61	0.05
22	6.52	6.63	0.11	6.63	0.11	6.64	0.12	6.64	0.12
23	7.48	7.62	0.14	7.63	0.15	7.62	0.14	7.61	0.13
28[b]	1.16	1.26	0.10	1.36	0.20	1.25	0.09	1.37	0.21
29[b]	0.83	0.95	0.12	0.98	0.15	0.96	0.13	0.98	0.15
30	8.05	8.62	0.57	8.19	0.14	8.63	0.58	8.20	0.15
MAE			0.16		0.12		0.16		0.12
RMSD			0.21		0.14		0.21		0.14
Max			0.57		0.25		0.58		0.22

[a] Chemical shifts deviation greater than 0.20 ppm are colored in red. [b] ^{1}H shifts of homotopic protons were averaged.

limitation of NMR in differentiating between such diastereomers based on shifts alone. The local environment in the fragment containing rings A and F allows for differentiation between the originally proposed structure (**3**) and its C3-epimer (**4**) based on NMR shifts, but fails to relate stereogenic centers in this fragment and fragment containing rings C and D. What aggravates the situation is that there are no useful NOE enhancements across the pyridine spacer. In cases like this, one should take into consideration other criteria, for example, calculations of ECD spectra or the biosynthetic origins of the natural products.

Given the putative biosynthesis of the xylogranatins, including the possible relationship to the mexicanolides,[5-6] the configuration of the stereocenter at C5 should be *S* as in diastereomer **4** and not *R* as in **5** and **6**.[13] Additional support for structure **4** came from the ECD computations (Figure S1) which made structure **6** less likely, as it possessed a prominent calculated negative band around 205 nm that is not observed in the experimental spectrum. The ECD spectrum calculated for structure **4** exhibited all positive bands analogous to the experimental spectrum.

Conclusion

In conclusion, NMR calculations were utilized in substantiating the structure misassignment of xylogranatin F as well as predicting the correct structure (**4**), which is a demonstration of the utility of NMR calculations for stereochemical assignment. The hybrid DFT-parametric DU8+ method has demonstrated superior accuracy, with MAE (δ ^{13}C) of 0.6 ppm for the revised structure significantly better than MAE of 1.2 ppm achieved with a more standard mPW1PW91/6-311+G(2d,p)//B3LYP/6-31+G(d,p)

approach. The DU8+ computations gave this superior accuracy at a fraction of time: calculations of chemical shifts per conformer took under 7 min on a 16-core node of a Linux cluster for DU8+, while the more conventional method, GIAO at the mPW1PW91/6-311+G(2d,p) level, on the same node took ten times longer, 70+ min. It is expected that the approach taken herein will help in the structural assignment of other limonoids.

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Supporting information

Additional supporting information including optimized structures, energies and ECD calculations is available in the online version of this article at the publisher's website.

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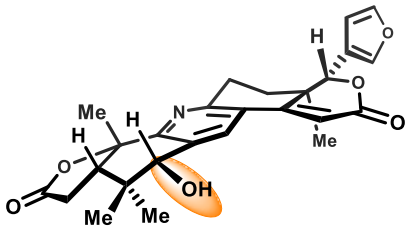
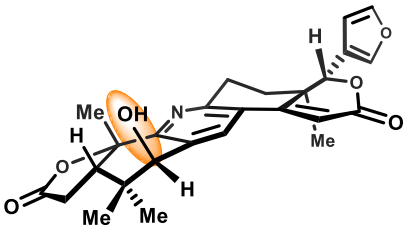


(+)-xylogranatin F
[proposed structure]



(+)-xylogranatin F
[revised structure]