# Supplementary data

# Tetrasubstituted *α*-pyrone derivatives from the endophytic fungus, *Neurospora udagawae*

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**Fungal Material**

*N. udagawae* isolate were characterized based on morphological criteria according to Garcia and co-workers.[1] Cultural characteristics of *N. udagawae*, including colony characters shape, and growth rate and microscopic characteristics including ascomata, asci and ultrastructure of ascospores, were recorded on PDA and OA.

Colonies on potato dextrose agar (PDA) and oatmeal agar (OA) attained a diameter of 80 mm within 4 days of incubation at 25. Colonies were flat to slightly raised on PDA and OA. Colony color on the surface and on the reverse of PDA and OA was olivaceous (Fig S1). Perithecia pyriform, ostiolate with periphysis, superficial, scattered to grouped, dark brown to blak, (450-) 600 (-850) × (330) 375 (-425) µm, with cylindrical to relatively conical neck, neck 125-250 × 100-180 µm. Peridium *textura angularis* to *textura globulosa*, 6-8 layered, 40-60 µm thick, outer layers 2-3, consisting of dark brown, thick-walled cells, 13–17 µm thick; middle layers 3-5, consisting of hyaline, thin-walled cells, 20-25 µm thick and inner layers 2-3, consisting of subhyaline, thin-walled to slightly thick wall cells, 14-18 µm. Asci consisting eight ascospores, thin-walled, cylindrical to subcylindrical, the spore-bearing part 150-200 × 15-18 µm, stipes 125-150 µm, with distinct apical ring, paraphyses not observed. Ascospores ellipsoidal to spindle shape, hyaline when young, becoming dark brown to black, (20-) 21.7-22.6 (-24) × (13-) 13.3-13.8 (-15) µm, consisting small circular pits 0.5 to 1.5 µm diam, one germ pore at each end (Fig S1).

**Identification of fungal culture**. For genomic DNA extraction, fresh fungal mycelia were harvested from 7 days old culture grown on MEA. Mycelia were subjected to DNA extraction using the EZ-10 Spin Column Genomic DNA Miniprep kit (Bio Basic Canada Inc., Markham, Ontario, Canada) following the manufacturer's protocol. Sequence data of internal transcribed spacer (ITS) regions was subjected to molecular analysis. Fragments were amplified using ITS1F/ITS4 primer (White et al., 1990). The amplicons were sequenced in both directions using the same primer set. The resulting sequence files were edited using SeqManTMII software in the Lasergene package (DNASTAR Inc., Madison, WI, USA) and consensus sequence was generated using the forward and reverse sequences. Basic local alignment search tool (BLAST) was conducted to compare consensus sequence with sequences in the GenBank. Consensus sequence of ITS-rDNA was deposited in GenBank with the accession number MK886745.

To determine the relationship of our isolate (CCTU A145) with known *Neurospora* species, ITS-rDNA sequence data of references strains were downloaded from GenBank and included in the alignment files. Bayesian analyses were carried out according to a previously published procedure.[2]

Megablast search analysis showed high similarity with reference sequence of *Neurospora* species from GenBank. A phylogeny was inferred based on ITS-rDNA sequence obtained in this study along with a reference sequence data from the GenBank. The final sequence alignment of the ITS-rDNA sequence comprising 25 internal taxa, had 553 characters and 106 unique site. *Achaetomium strumarium* CBS 333.67 (AY681204) served as the outgroup taxon. Bayesian analyses were performed using the best-fitting substitution (SYM+G) model and resulted in 542 generations. After discarding the first 25% of generations considered as burn-in, the remaining 408 (75%) generations were used to calculate the consensus Bayesian tree and posterior probabilities. Results indicated that the isolate used in this study clustered together with *Neurospora* species in same clade with highly supported value (Fig S2) .

Previous researches revealed that ornamentation pattern of the surface of ascospores, is an informative character. According to a combination of morphological feature, especially ultrastructure of ascospores and phylogenetic data, the isolate was identified as *Neurospora udagawae*.

**References**


1. García, D., Stchigel, A. M., José, C. A., Guarro, J., Hawksworth, D. L. 2004. A synopsis and re-circumscription of *Neurospora* (syn. *Gelasinospora*) based on ultrastructural and 28S rDNA sequence data. Mycol. Res. 108, 1119–1142.
2. Narmani, A., Pichai, S., Palani, P., Arzanlou, M., Surup, F., Stadler, M., 2018. Daldinia sacchari (Hypoxylaceae) from India produces the new cytochalasins Saccalasins A and B and belongs to the D. eschscholtzii species complex. Mycol. Prog. https://doi.org/10.1007/s11557-018-1413-6.
3. Cheng, T., Chepkirui, C., Decock, C., Matasyoh, J. C., Stadler, M. 2019. Sesquiterpenes from an eastern African medicinal mushroom belonging to the genus Sanghuangporus. J. Nat. Prod. *in press*.

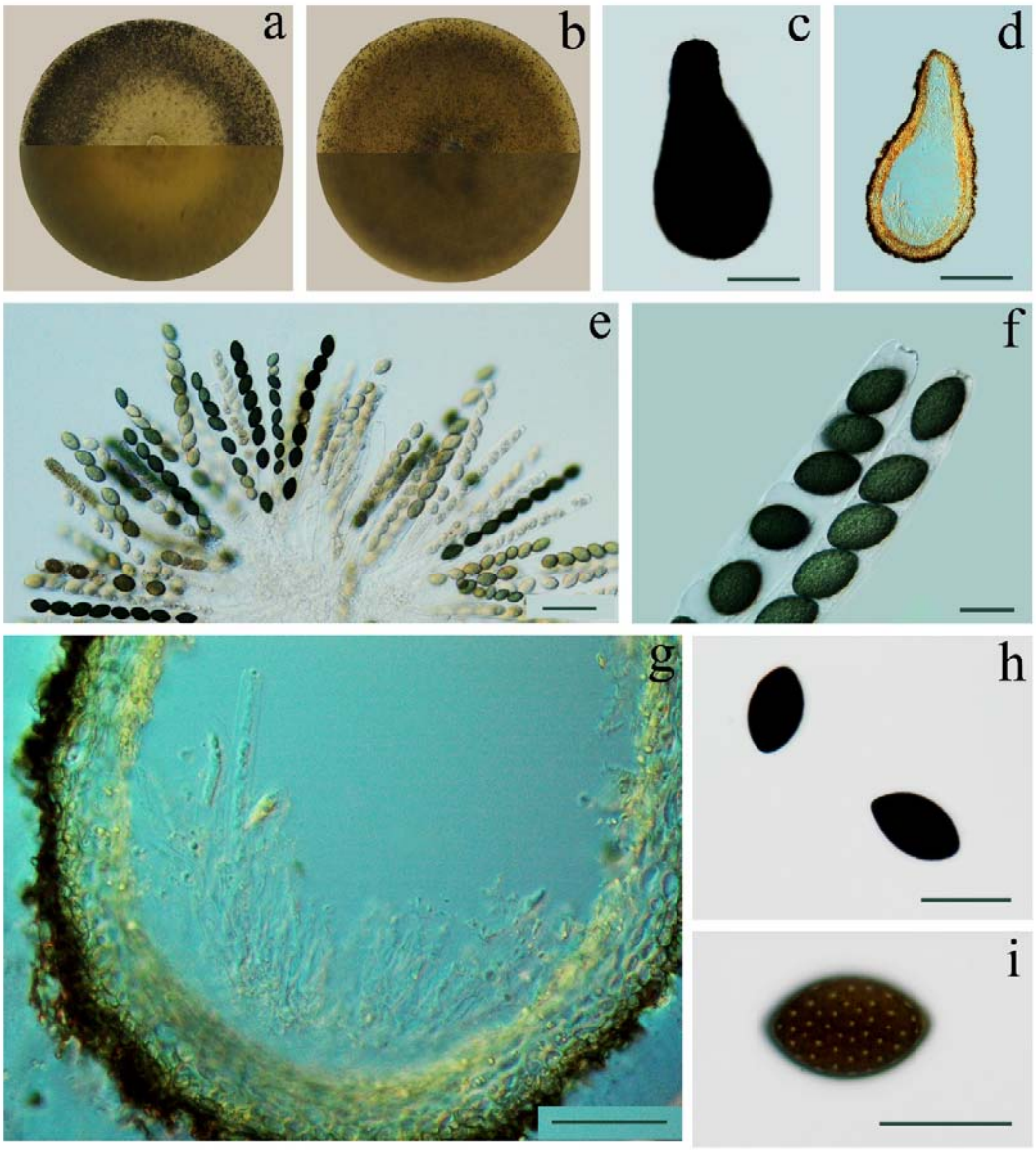


**Fig. S1** Morphological characteristics of *Neurospora udagawae*. a-b- 14 days old culture on the surface and on the reverse on PDA and OA, respectively, c- ascomata, d- longitudinal section of ascomata, e-f- asci consisting eight ascospores, g- Peridium of ascomata, h- ascospores, i- ornamentation pattern of the surface of their ascospores. Scale bars: c, f = 200 µm, e,g= 50 µm g, f, h, i = 20 µm.

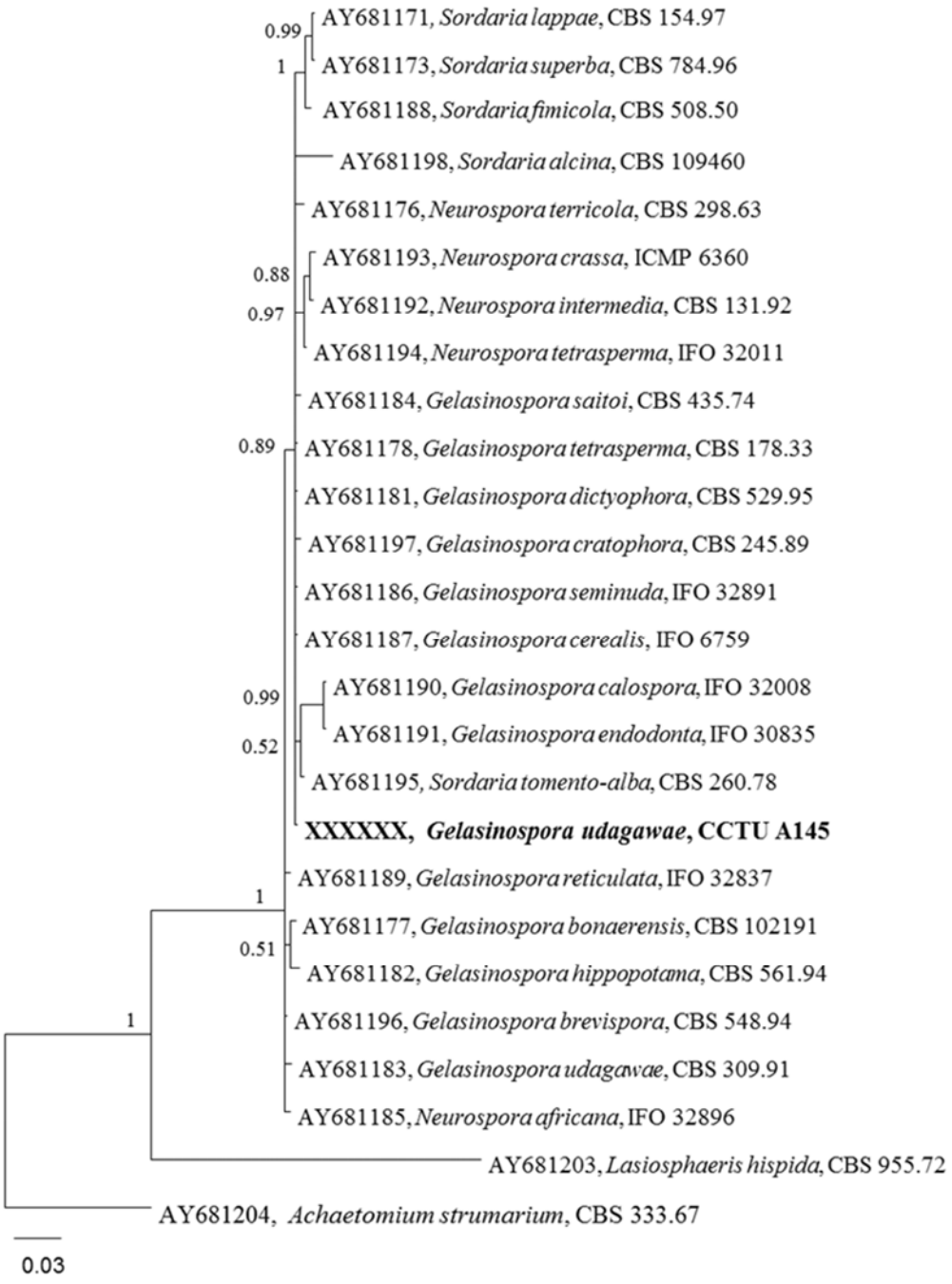


**Fig. S2** Consensus phylogram (75% majority rule) of 408 trees resulting from a Bayesian analysis of ITS-rDNA region sequence alignment using MrBayes v. 3.2.2. The scale bar indicates 0.03 expected changes per site. The tree was rooted to *Achaetomium strumarium* CBS 333.67 (AY681204).

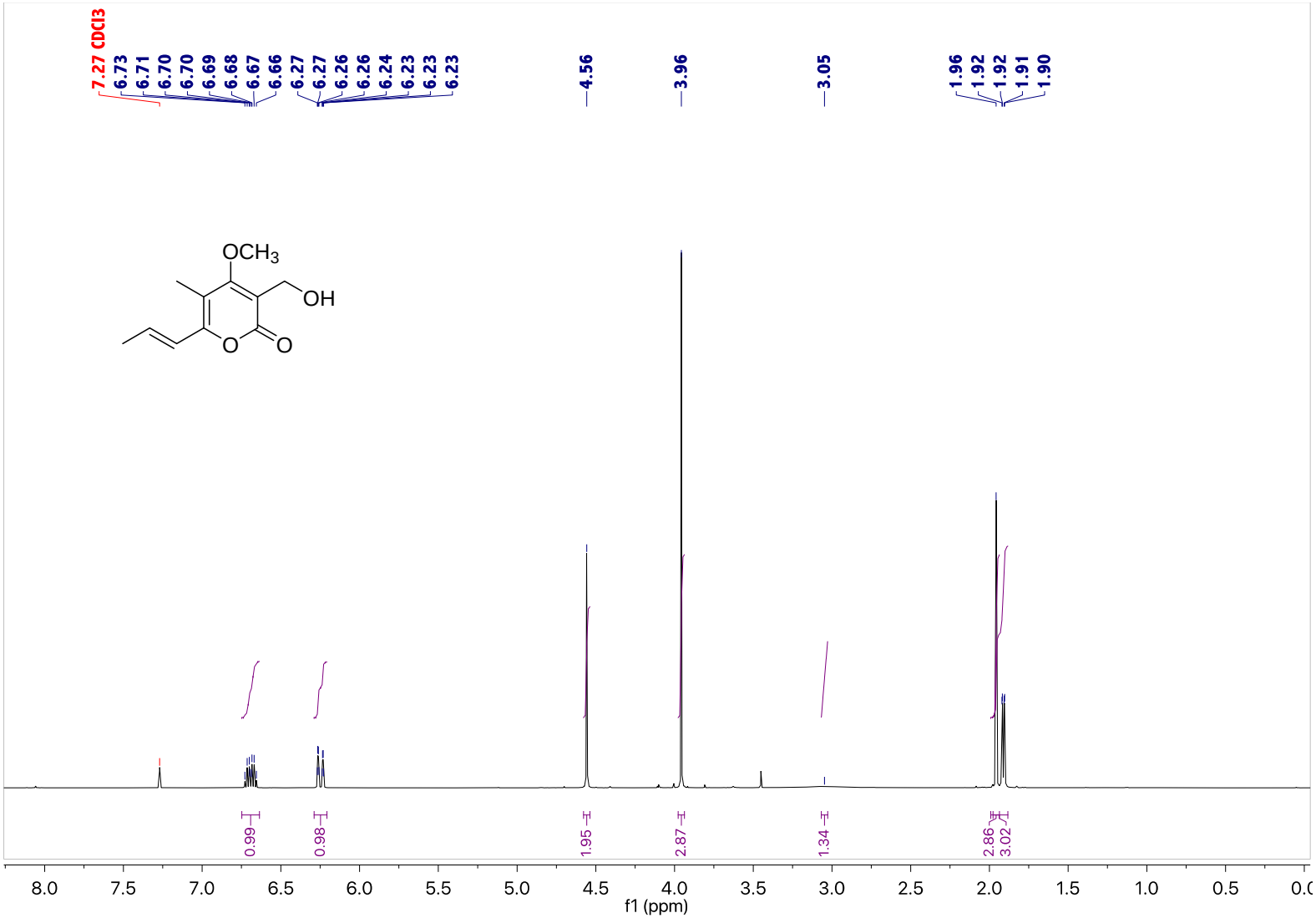


**Fig. S3.** $^{1}$H NMR spectrum of udagawanone A (**1**) (500 MHz, $CDCl_3$).

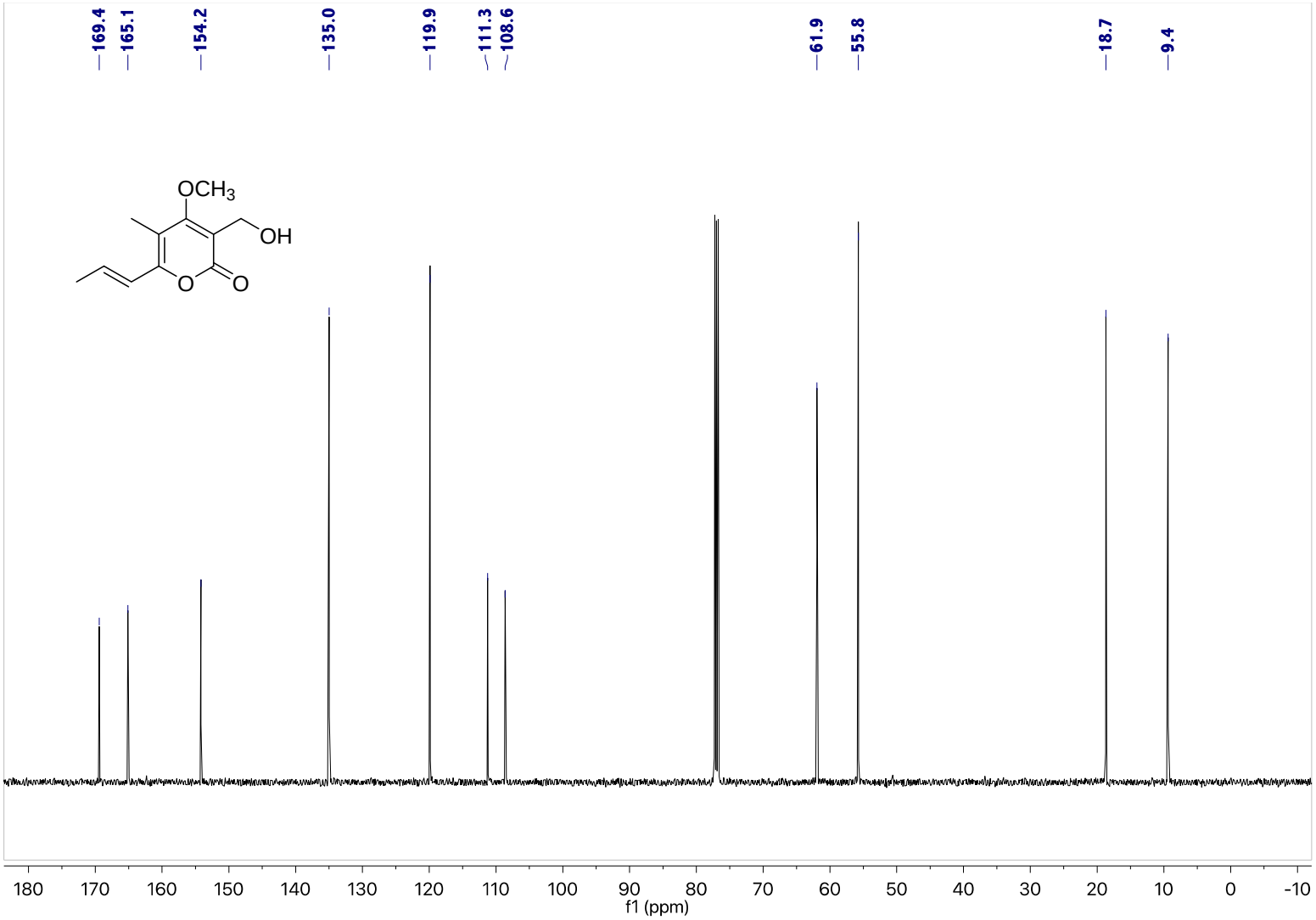


**Fig. S4.** $^{13}C$ NMR spectrum of udagawanone A (**1**) (125 MHz, $CDCl_3$).

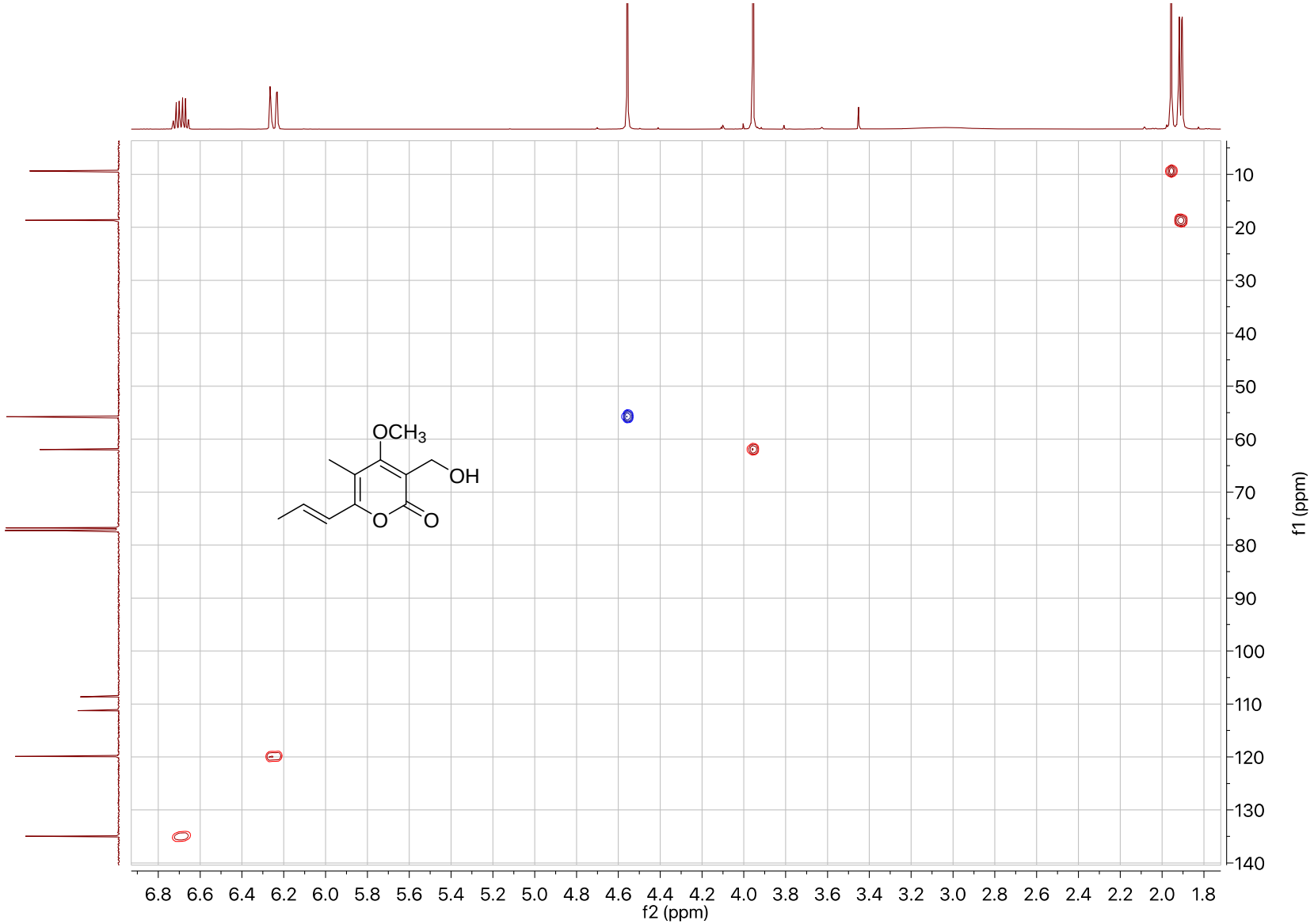


**Fig. S5.** HSQC spectrum of udagawanone A (**1**).

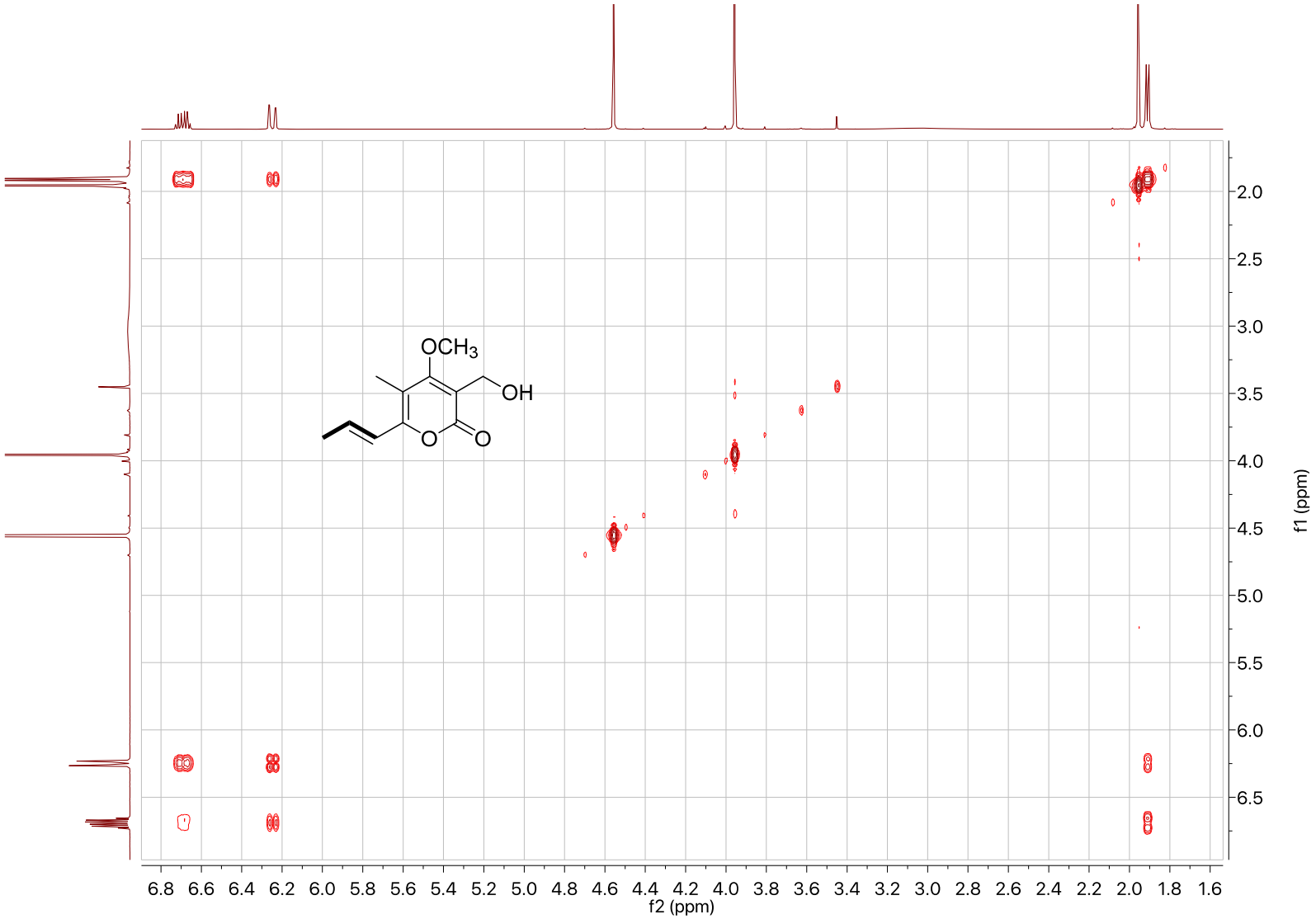


**Fig. S6.** COSY spectrum of udagawanone A (**1**).

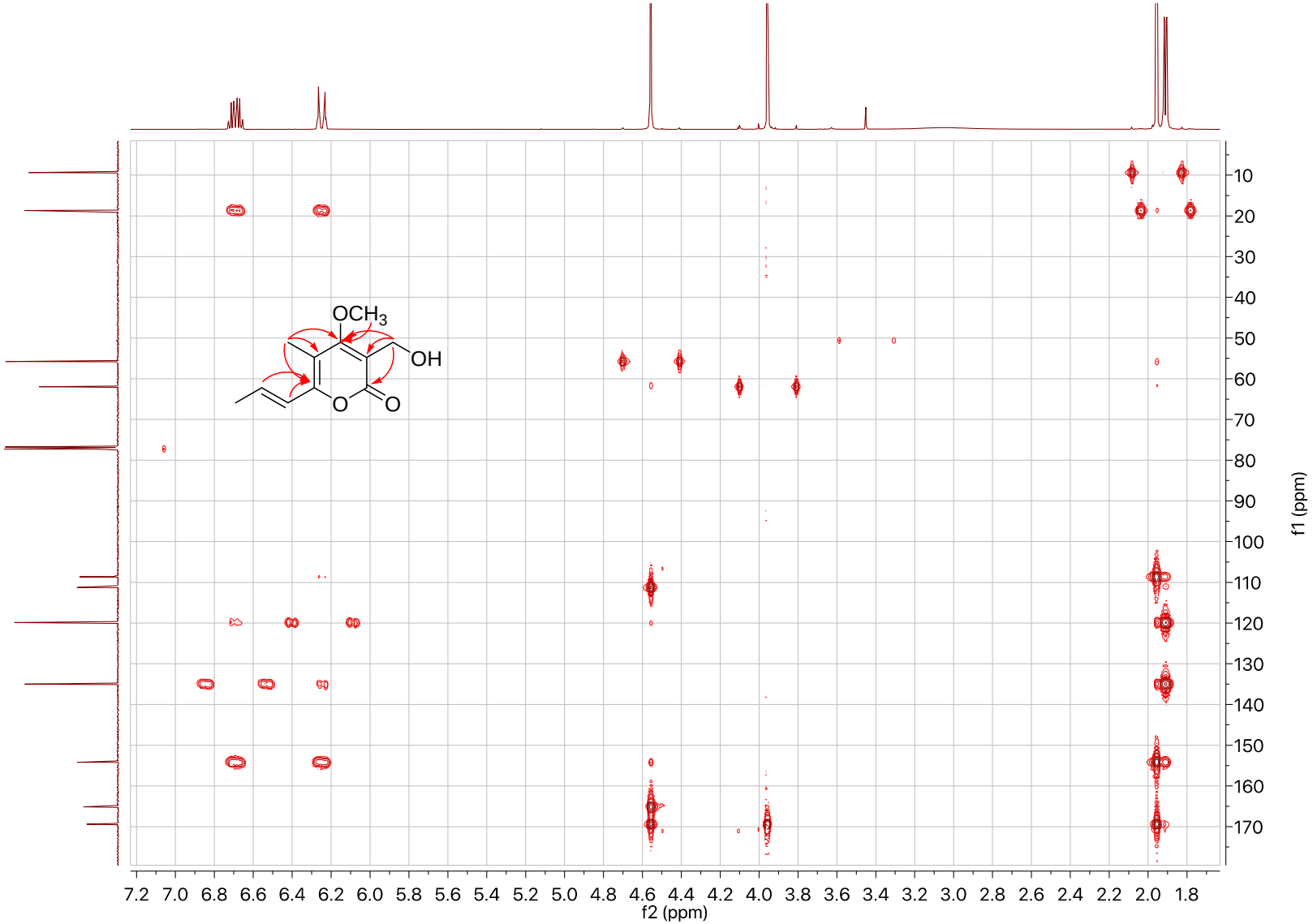


**Fig. S7.** HMBC spectrum of udagawanone A (**1**).

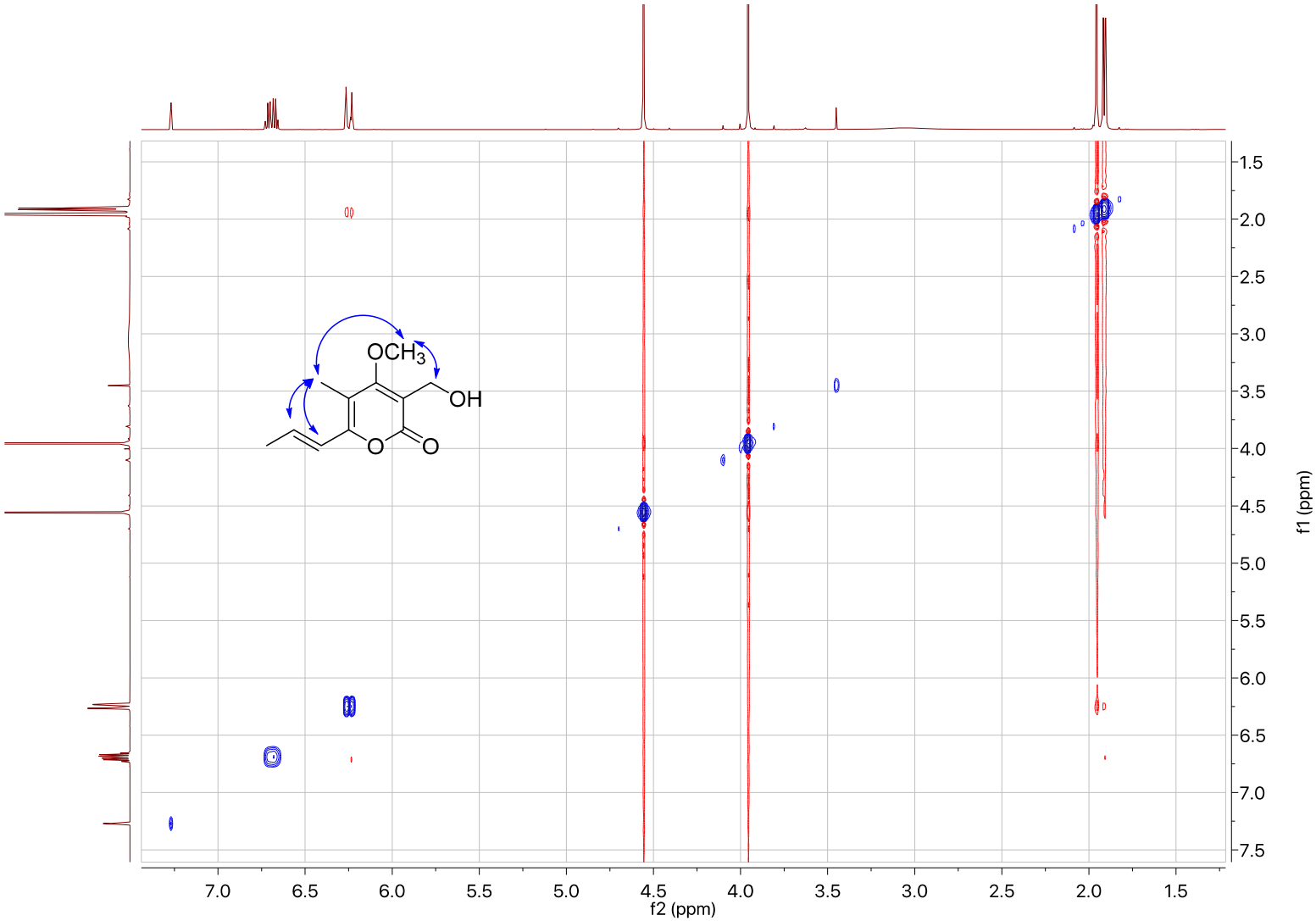


**Fig. S8.** ROESY spectrum of udagawanone A (**1**).

## X-ray data of udagawanone A (1)

**Table S1.** Crystal data and structure refinement for **1**.

| | |
|---|---|
| Identification code | sh4066 |
| Empirical formula | C11 H14 O4 |
| Formula weight | 210.22 |
| Temperature | 132(2) K |
| Wavelength | 0.71073 Å |
| Crystal system | Triclinic |
| Space group | P-1 |
| Unit cell dimensions | a = 7.2824(5) Å α= 100.6450(18)°. |
| b = 8.3992(5) Å | β= 105.6283(18)°. |
| c = 9.1212(6) Å | γ = 103.0011(18)°. |
| Volume | 505.30(6) $Å^3$ |
| Z | 2 |
| Density (calculated) | 1.382 $Mg/m^3$ |
| Absorption coefficient | 0.105 $mm^{-1}$ |
| F(000) | 224 |
| Crystal size | 0.499 x 0.357 x 0.122 $mm^3$ |
| Theta range for data collection | 2.402 to 29.608°. |
| Index ranges | -10<=h<=10, -11<=k<=11, -12<=l<=12 |
| Reflections collected | 15249 |
| Independent reflections | 2840 [R(int) = 0.0248] |
| Completeness to theta = 25.242° | 100.0 % |
| Absorption correction | Semi-empirical from equivalents |
| Max. and min. transmission | 0.7459 and 0.7298 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 2840 / 0 / 192 |
| Goodness-of-fit on $F^2$ | 1.079 |
| Final R indices [I>2sigma(I)] | R1 = 0.0355, wR2 = 0.0924 |
| R indices (all data) | R1 = 0.0478, wR2 = 0.1003 |
| Extinction coefficient | n/a |
| Largest diff. peak and hole | 0.351 and -0.220 $e.Å^{-3}$ |

**Table S2.** Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters ($Å^2$x $10^3$) for sh4066. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| O(1) | 2421(1) | 1014(1) | 8381(1) | 20(1) |
| O(2) | 2871(1) | 3758(1) | 9050(1) | 25(1) |
| O(3) | 3009(1) | 489(1) | 12827(1) | 23(1) |
| O(4) | 5100(1) | 5058(1) | 12944(1) | 32(1) |
| C(1) | 2744(1) | 2470(1) | 9506(1) | 19(1) |
| C(2) | 2889(1) | 2376(1) | 11089(1) | 18(1) |
| C(3) | 2826(1) | 838(1) | 11424(1) | 17(1) |
| C(4) | 2508(1) | -669(1) | 10206(1) | 17(1) |
| C(5) | 2296(1) | -521(1) | 8720(1) | 17(1) |
| C(6) | 3089(2) | 4040(1) | 12177(1) | 25(1) |
| C(7) | 3759(2) | 1790(1) | 14295(1) | 24(1) |
| C(8) | 2412(2) | -2318(1) | 10640(1) | 21(1) |
| C(9) | 1920(2) | -1868(1) | 7321(1) | 20(1) |
| C(10) | 1424(2) | -1684(1) | 5858(1) | 22(1) |
| C(11) | 1012(2) | -3048(2) | 4406(1) | 29(1) |

**Table S3.** Bond lengths [Å] and angles [°] for **1**.

| | |
|---|---|
| O(1)-C(5) | 1.3694(11) |
| O(1)-C(1) | 1.3714(11) |
| O(2)-C(1) | 1.2229(12) |
| O(3)-C(3) | 1.3442(12) |
| O(3)-C(7) | 1.4430(12) |
| O(4)-C(6) | 1.4244(14) |
| O(4)-H(3) | 0.846(16) |
| C(1)-C(2) | 1.4372(14) |
| C(2)-C(3) | 1.3745(13) |
| C(2)-C(6) | 1.5095(14) |
| C(3)-C(4) | 1.4487(13) |
| C(4)-C(5) | 1.3548(13) |
| C(4)-C(8) | 1.5008(13) |
| C(5)-C(9) | 1.4570(13) |
| C(6)-H(1) | 0.995(14) |
| C(6)-H(2) | 0.991(13) |
| C(7)-H(4) | 0.977(13) |
| C(7)-H(5) | 0.983(14) |
| C(7)-H(6) | 0.990(14) |
| C(8)-H(7) | 0.961(15) |
| C(8)-H(8) | 0.986(15) |
| C(8)-H(9) | 0.940(15) |
| C(9)-C(10) | 1.3327(14) |
| C(9)-H(10) | 0.977(14) |
| C(10)-C(11) | 1.4920(15) |
| C(10)-H(11) | 0.943(15) |
| C(11)-H(12) | 0.970(16) |
| C(11)-H(13) | 0.976(17) |
| C(11)-H(14) | 0.994(15) |
| C(5)-O(1)-C(1) | 122.42(8) |
| C(3)-O(3)-C(7) | 122.70(8) |
| C(6)-O(4)-H(3) | 108.5(10) |
| O(2)-C(1)-O(1) | 115.62(9) |
| O(2)-C(1)-C(2) | 125.46(9) |

| | |
|---|---|
| O(1)-C(1)-C(2) | 118.92(8) |
| C(3)-C(2)-C(1) | 117.93(9) |
| C(3)-C(2)-C(6) | 128.63(9) |
| C(1)-C(2)-C(6) | 113.44(9) |
| O(3)-C(3)-C(2) | 127.51(9) |
| O(3)-C(3)-C(4) | 111.05(8) |
| C(2)-C(3)-C(4) | 121.44(9) |
| C(5)-C(4)-C(3) | 118.05(8) |
| C(5)-C(4)-C(8) | 123.05(9) |
| C(3)-C(4)-C(8) | 118.90(8) |
| C(4)-C(5)-O(1) | 121.11(8) |
| C(4)-C(5)-C(9) | 127.17(9) |
| O(1)-C(5)-C(9) | 111.72(8) |
| O(4)-C(6)-C(2) | 113.51(9) |
| O(4)-C(6)-H(1) | 111.0(8) |
| C(2)-C(6)-H(1) | 106.3(8) |
| O(4)-C(6)-H(2) | 106.1(8) |
| C(2)-C(6)-H(2) | 112.4(7) |
| H(1)-C(6)-H(2) | 107.5(11) |
| O(3)-C(7)-H(4) | 110.1(7) |
| O(3)-C(7)-H(5) | 109.5(8) |
| H(4)-C(7)-H(5) | 111.8(11) |
| O(3)-C(7)-H(6) | 103.3(8) |
| H(4)-C(7)-H(6) | 111.6(11) |
| H(5)-C(7)-H(6) | 110.3(11) |
| C(4)-C(8)-H(7) | 110.5(9) |
| C(4)-C(8)-H(8) | 111.4(8) |
| H(7)-C(8)-H(8) | 108.7(12) |
| C(4)-C(8)-H(9) | 112.1(9) |
| H(7)-C(8)-H(9) | 107.6(12) |
| H(8)-C(8)-H(9) | 106.3(12) |
| C(10)-C(9)-C(5) | 123.79(9) |
| C(10)-C(9)-H(10) | 119.8(8) |
| C(5)-C(9)-H(10) | 116.4(8) |
| C(9)-C(10)-C(11) | 124.81(10) |
| C(9)-C(10)-H(11) | 117.9(9) |

| | |
|---|---|
| C(11)-C(10)-H(11) | 117.2(9) |
| C(10)-C(11)-H(12) | 109.7(9) |
| C(10)-C(11)-H(13) | 110.7(9) |
| H(12)-C(11)-H(13) | 106.5(13) |
| C(10)-C(11)-H(14) | 111.2(8) |
| H(12)-C(11)-H(14) | 107.7(12) |
| H(13)-C(11)-H(14) | 110.9(13) |

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Symmetry transformations used to generate equivalent atoms:

**Table S4.** Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **1**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| O(1) | 25(1) | 16(1) | 17(1) | 5(1) | 6(1) | 5(1) |
| O(2) | 31(1) | 18(1) | 26(1) | 9(1) | 9(1) | 6(1) |
| O(3) | 35(1) | 19(1) | 16(1) | 4(1) | 10(1) | 5(1) |
| O(4) | 44(1) | 22(1) | 21(1) | 1(1) | 11(1) | -4(1) |
| C(1) | 17(1) | 17(1) | 21(1) | 4(1) | 6(1) | 4(1) |
| C(2) | 18(1) | 17(1) | 20(1) | 3(1) | 7(1) | 5(1) |
| C(3) | 16(1) | 18(1) | 17(1) | 4(1) | 6(1) | 4(1) |
| C(4) | 16(1) | 15(1) | 18(1) | 4(1) | 6(1) | 4(1) |
| C(5) | 16(1) | 15(1) | 19(1) | 4(1) | 5(1) | 4(1) |
| C(6) | 36(1) | 17(1) | 24(1) | 4(1) | 13(1) | 8(1) |
| C(7) | 29(1) | 23(1) | 16(1) | 1(1) | 7(1) | 5(1) |
| C(8) | 30(1) | 16(1) | 19(1) | 6(1) | 8(1) | 8(1) |
| C(9) | 21(1) | 19(1) | 18(1) | 3(1) | 6(1) | 5(1) |
| C(10) | 23(1) | 24(1) | 20(1) | 5(1) | 7(1) | 5(1) |
| C(11) | 31(1) | 34(1) | 17(1) | 2(1) | 6(1) | 5(1) |

Table S5. Hydrogen coordinates ( x $10^4$) and isotropic displacement parameters ($Å^2$x $10^3$) for **1**.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| H(3) | 5570(20) | 5377(18) | 12257(18) | 37(4) |
| H(1) | 2310(20) | 4634(18) | 11517(16) | 33(4) |
| H(2) | 2514(19) | 3887(16) | 13027(16) | 26(3) |
| H(4) | 4802(19) | 2725(17) | 14254(15) | 25(3) |
| H(5) | 2650(20) | 2168(17) | 14512(16) | 29(3) |
| H(6) | 4290(20) | 1199(17) | 15081(17) | 31(3) |
| H(7) | 3560(20) | -2201(18) | 11516(18) | 34(4) |
| H(8) | 1210(20) | -2718(18) | 10923(17) | 34(4) |
| H(9) | 2360(20) | -3184(18) | 9801(17) | 35(4) |
| H(10) | 2030(20) | -2959(18) | 7496(16) | 30(3) |
| H(11) | 1280(20) | -627(18) | 5722(17) | 35(4) |
| H(12) | 1820(20) | -2647(19) | 3787(18) | 42(4) |
| H(13) | -370(20) | -3337(19) | 3739(19) | 44(4) |
| H(14) | 1350(20) | -4064(19) | 4675(17) | 36(4) |

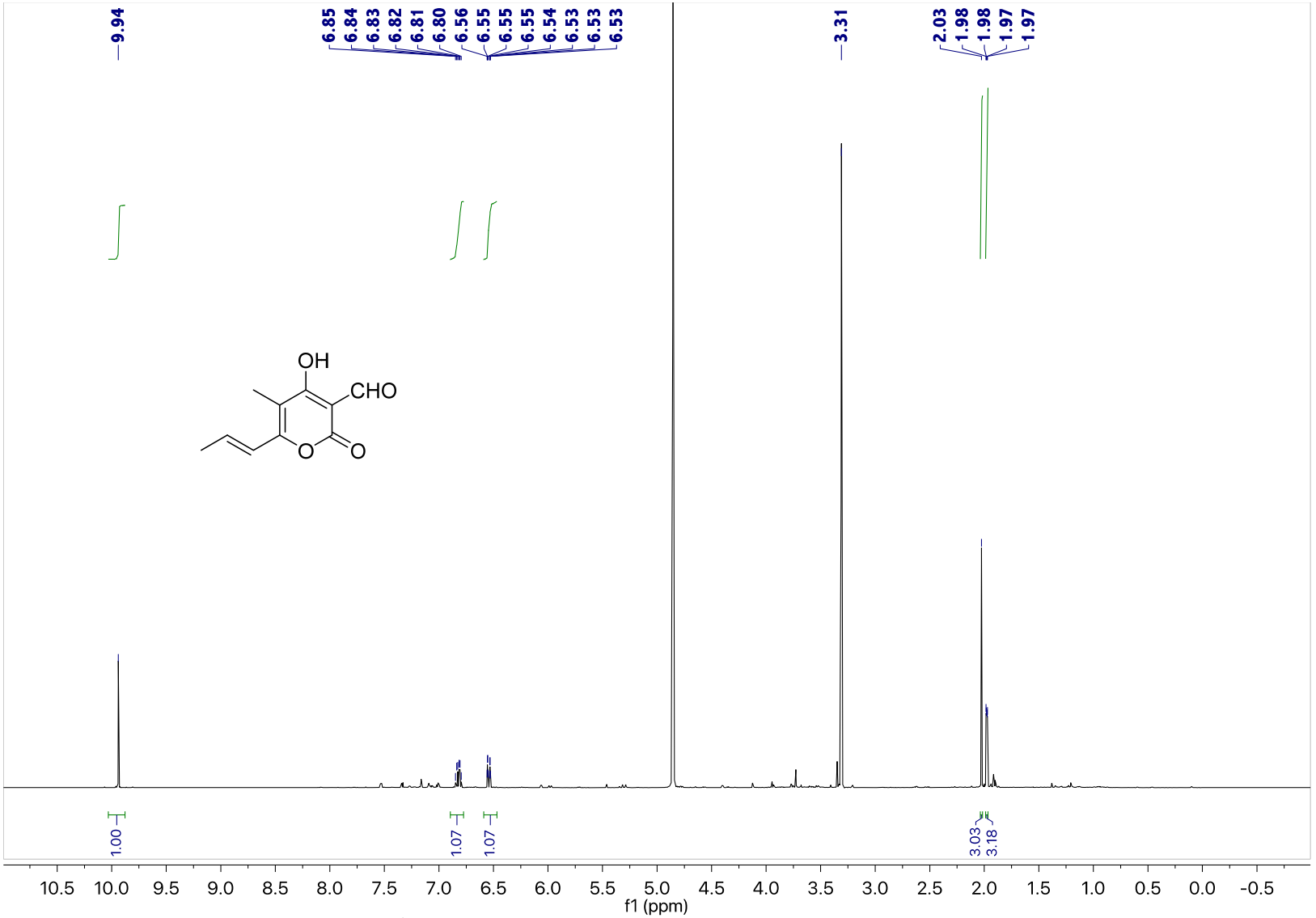


**Fig. S9.** $^{1}$H NMR spectrum of udagawanone B (**2**) (700 MHz, MeOH-$d_4$).

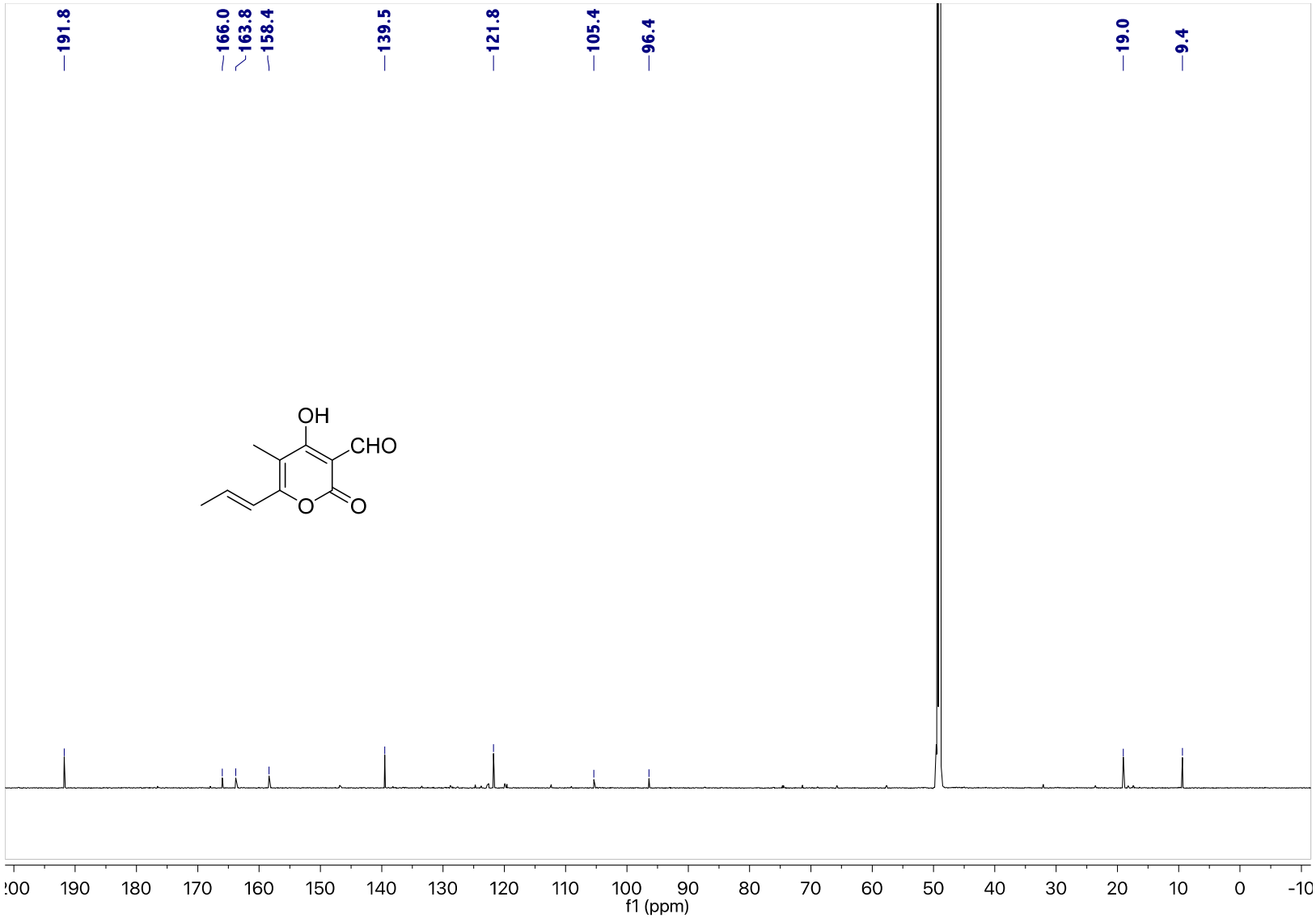


**Fig. S10.** $^{13}$C NMR spectrum of udagawanone B (**2**) (175 MHz, MeOH-$d_4$).

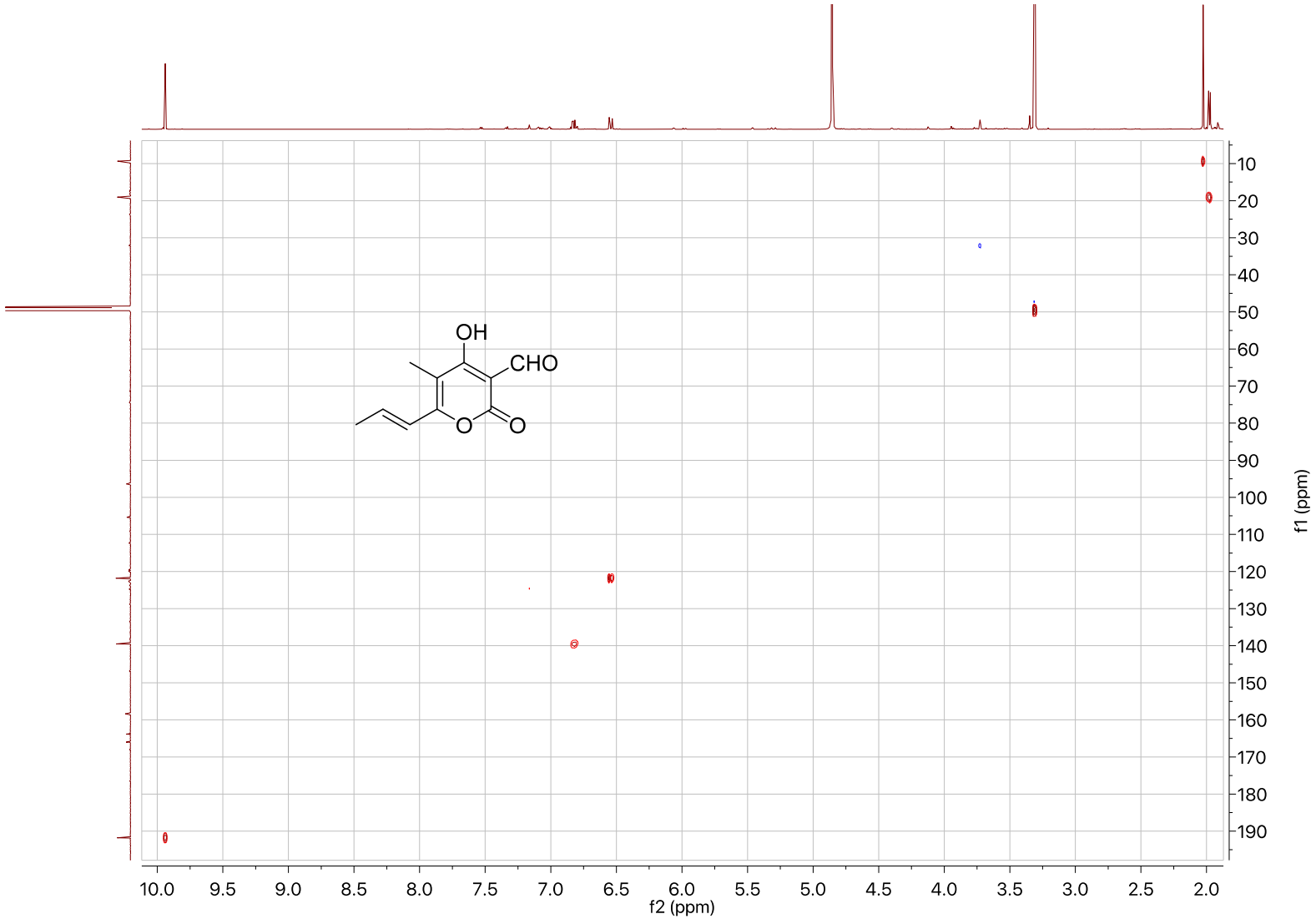


**Fig. S11.** HSQC spectrum of udagawanone B (**2**).

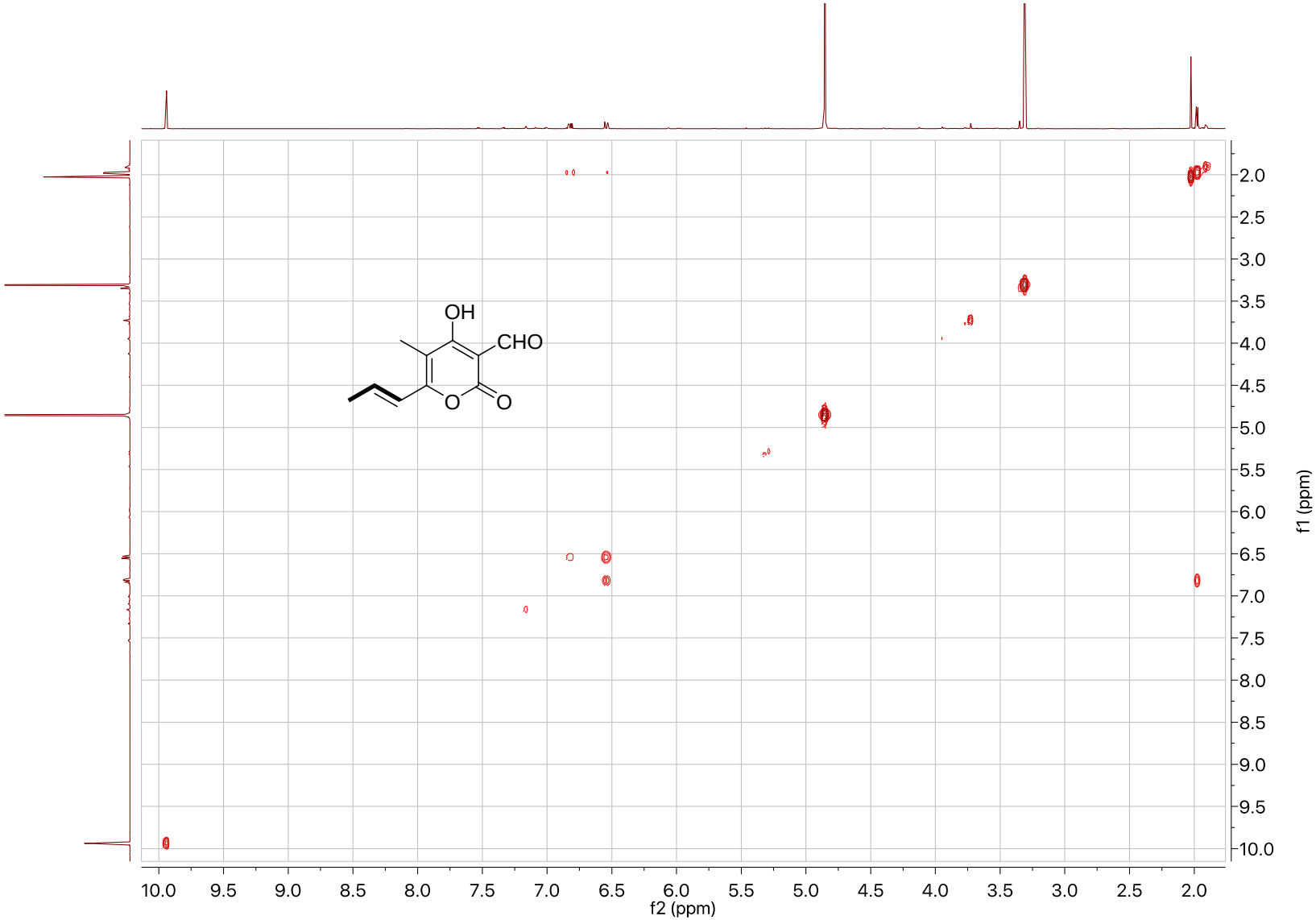


**Fig. S12.** COSY spectrum of udagawanone B (**2**).

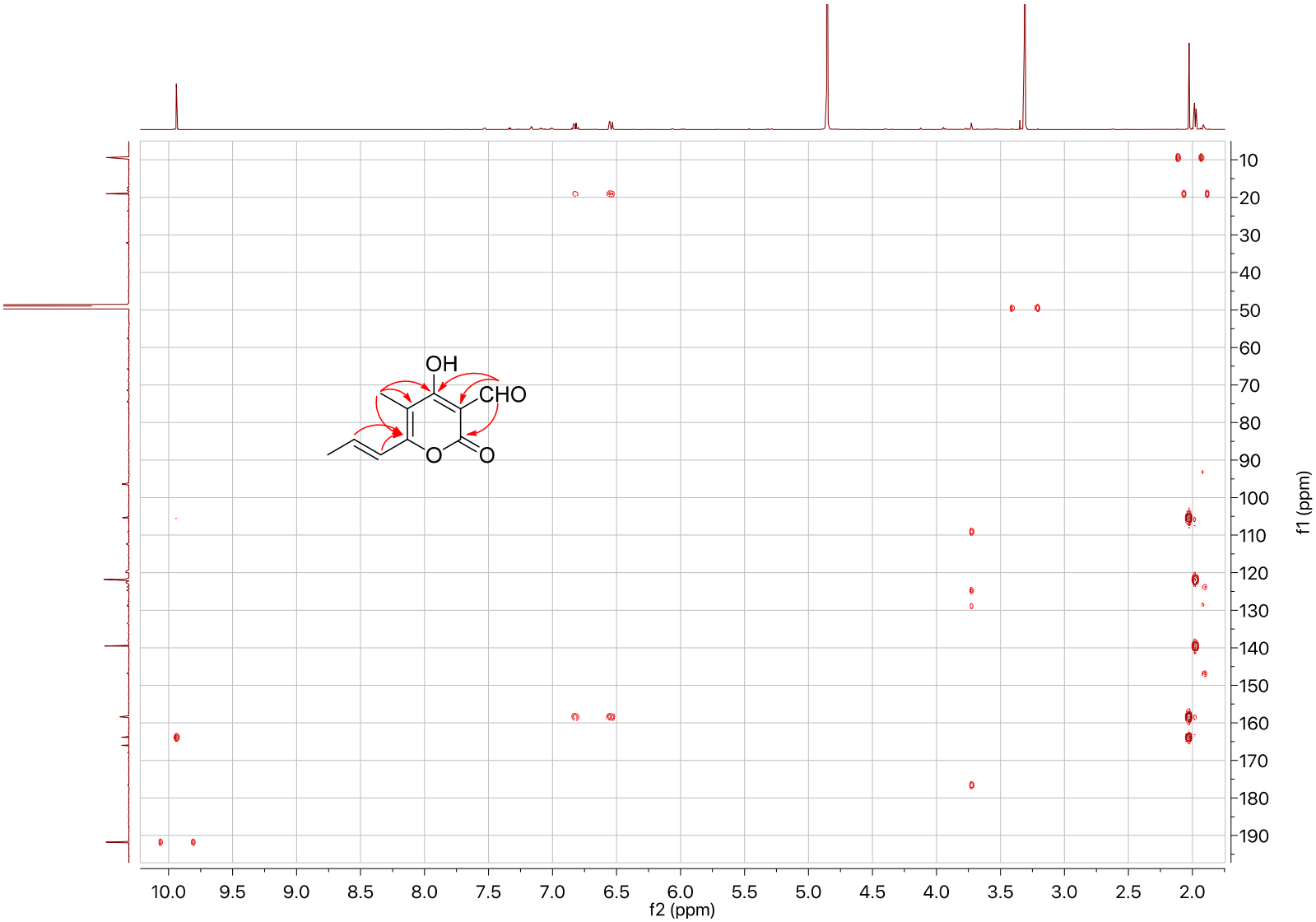


**Fig. S13.** HMBC spectrum of udagawanone B (**2**).

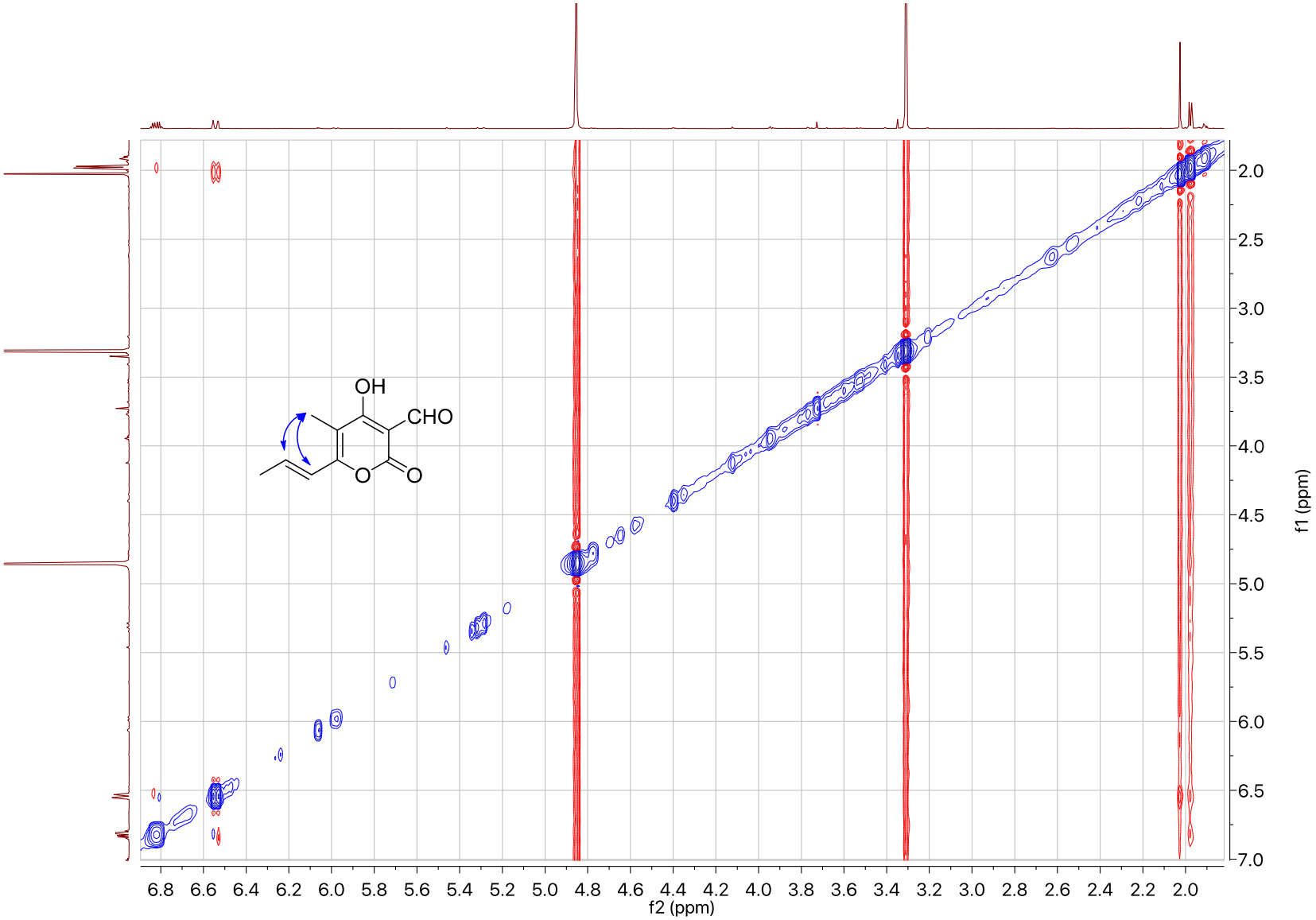


**Fig. S14.** ROESY spectrum of udagawanone B (**2**).