Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

An NMR Crystallographic Characterisation of Solid (+)-Usnic Acid

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Supporting Information

S1. Further Details of Solid-State NMR Experiments

Solid-state NMR experiments were recorded using Bruker Avance III spectrometers equipped with 9.4 T or 14.1 T wide-bore superconducting magnets (respective ^{1}H Larmor frequencies of 400.1 and 600.1 MHz, and respective ^{13}C Larmor frequencies of 100.6 and 150.9 MHz). Samples were packed into standard 4 mm or 1.3 mm zirconia MAS rotors. For the variable-temperature experiments, the sample temperature was controlled using a Bruker BVT control unit, BVTB-3000 heater booster and BCU-II chiller.

The ^{1}H NMR spectrum of (+)-usnic acid was recorded at 14.1 T with a MAS rate of 60 kHz. The DEPTH pulse sequence[S1] was used to minimise probe background. Signal averaging was carried out for 16 transients with a recycle interval of 120 s (note that signals may be absent or inverted in a DEPTH spectrum if the recycle interval is insufficient).

The ^{13}C NMR spectrum of (+)-usnic acid was recorded at 9.4 T with a MAS rate of 12.5 kHz using cross polarisation (CP) from ^{1}H with a contact pulse (ramped for ^{1}H) of 5 ms. Signal averaging was carried out for 64 transients with a recycle interval of 15 s. The ^{13}C CP MAS NMR spectrum of *U. dasopoga* powder was recorded with a contact pulse (ramped for ^{1}H) of 3 ms. Signal averaging was carried out for 4096 transients with a recycle interval of 15 s. Note that for both samples the rotor was not completely full. High-power ($\nu_1 \approx 100$ kHz) TPPM-15 decoupling of ^{1}H was applied during acquisition in both cases. We note as an aside the importance of correctly setting the magic angle to ensure the maximum possible resolution, especially for the carbonyl signals. This was particularly important for carbon 1, which has a shift difference of 0.4 ppm (40 Hz at 9.4 T) and, with a correctly set magic angle, linewidths of around 0.3 ppm. The magic angle was set by the standard method of minimising the width of the ^{79}Br spinning sidebands of KBr.

^{13}C CP-INADEQUATE NMR experiments were carried out at 9.4 T with a MAS rate of 12.5 kHz using a refocused pulse sequence[S2,S3] with initial ^{13}C magnetisation created by CP from ^{1}H using a contact pulse (ramped for ^{1}H) of 5 ms. For the experiment shown in the main text, a rotor-synchronised mixing time of 48 τ_r (= 3.84 ms) was used and for the spectra shown in section S3, additional mixing times of 40 and 56 t_r (= 3.2 and 4.48 ms) were used. Signal averaging was carried out for 1152 transients for each of 66-100 t_1 increments of 40 μs (= $\tau_r/2$) with a recycle interval of 3 s. The States method of quadrature detection was used for the indirect dimension. Spectra are processed with exponential weighting of 35 Hz in δ_2 and 80 Hz in δ_1.

The ^{1}H-^{13}C INEPT experiment was recorded at 14.1 T with a MAS rate of 60 kHz using a refocused pulse sequence. Signal averaging was carried out for 256 transients for each of 256 t_1 increments of 16.67 μs (= τ_r) with a recycle interval of 3 s and using the States method of quadrature detection in the indirect dimension.

The ^{1}H-^{13}C CP HETCOR experiment was recorded at 9.4 T with a MAS rate of 12.5 kHz using a contact pulse (ramped for ^{1}H) of 350 μs and a recycle interval of 3 s. Signal averaging was carried out for 48 transients for each of 48 t_1 increments of 80 μs (= τ_r) using the States method of quadrature detection in the indirect dimension. The spectrum shown was processed with exponential weighting of 25 Hz for ^{13}C and 50 Hz for ^{1}H.

The ^{1}H-^{1}H SQ-DQ correlation experiment was recorded at 14.1 T with a MAS rate of 60 kHz and using the BABA pulse scheme to excite and convert double quantum coherences.[S4] Two blocks of BABA pulses were used for excitation and conversion. Signal averaging was carried out for 64 transients for each of 150 t_1 increments of 16.67 μs (= τ_r) with a recycle interval of 3 s. Quadrature detection in the indirect dimension was achieved using the States method.

For the variable-temperature experiments, each measurement was recorded using a CP sequence with a contact pulse (ramped for ^{1}H) of 5 ms and signal averaging for 1024 transients with a recycle interval of 5 s. Prior to acquisition, the temperature was allowed to stabilise to within ±0.1 K and then equilibrated for 300 s. Note that the probe detuned during the overnight VT run and the intensities of some signals were very poor despite the extensive signal averaging.

S2. Complete One-Dimensional NMR Spectra

Figure S1 shows the complete ^{13}C DEPTQ spectrum of (+)-usnic acid in $CDCl_3$ and the complete ^{13}C CP MAS NMR spectrum of solid (+)-usnic acid.

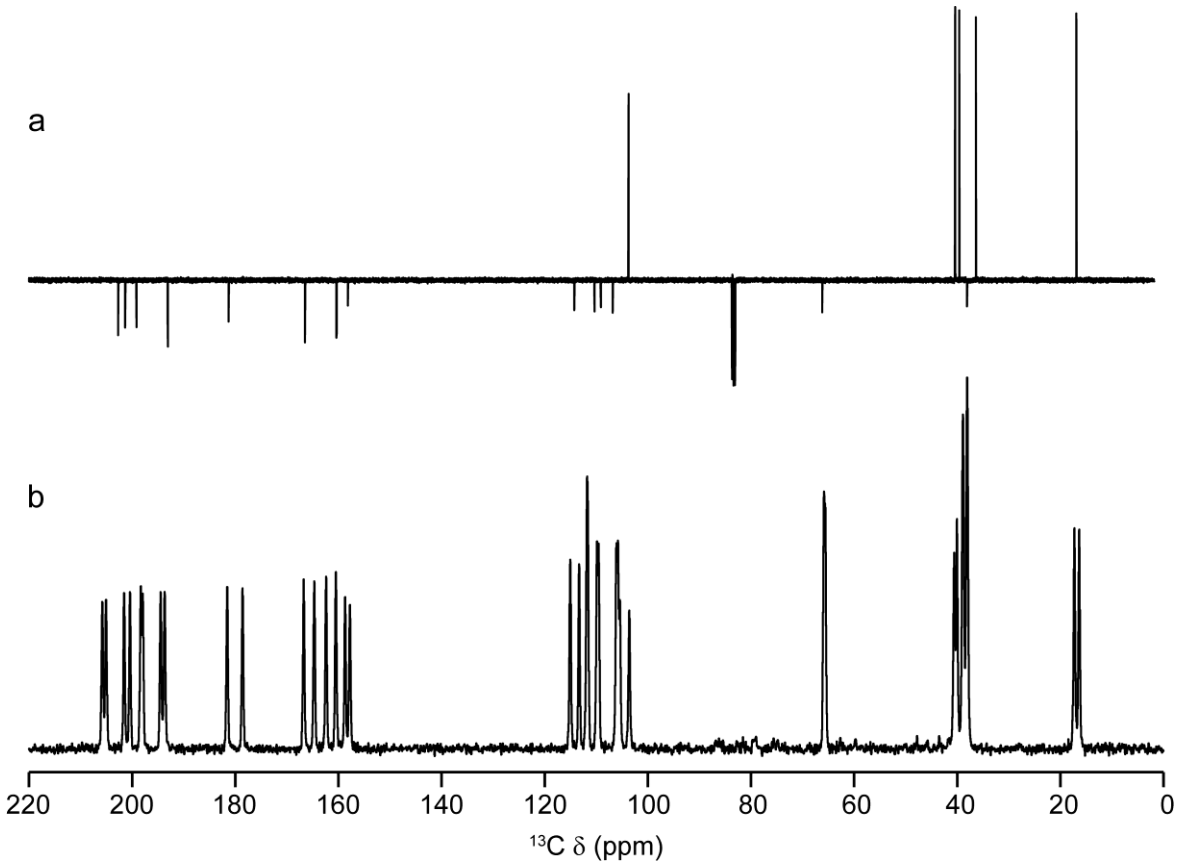

Figure S1. (a) ^{13}C DEPTQ NMR spectrum of (+)-usnic acid (9.4 T, $CDCl_3$; CH_3 and CH positive, CH_2 and C negative). (b) ^{13}C CP MAS NMR spectrum of solid (+)-usnic acid (9.4 T, 12.5 kHz MAS).

S3. Additional ^{13}C INADEQUATE NMR Spectra

Figures S2-S4 show ^{13}C CP-INADEQUATE NMR spectra of (+)-usnic acid recorded with J evolution times of 3.2, 3.84 and 4.48 ms, respectively.

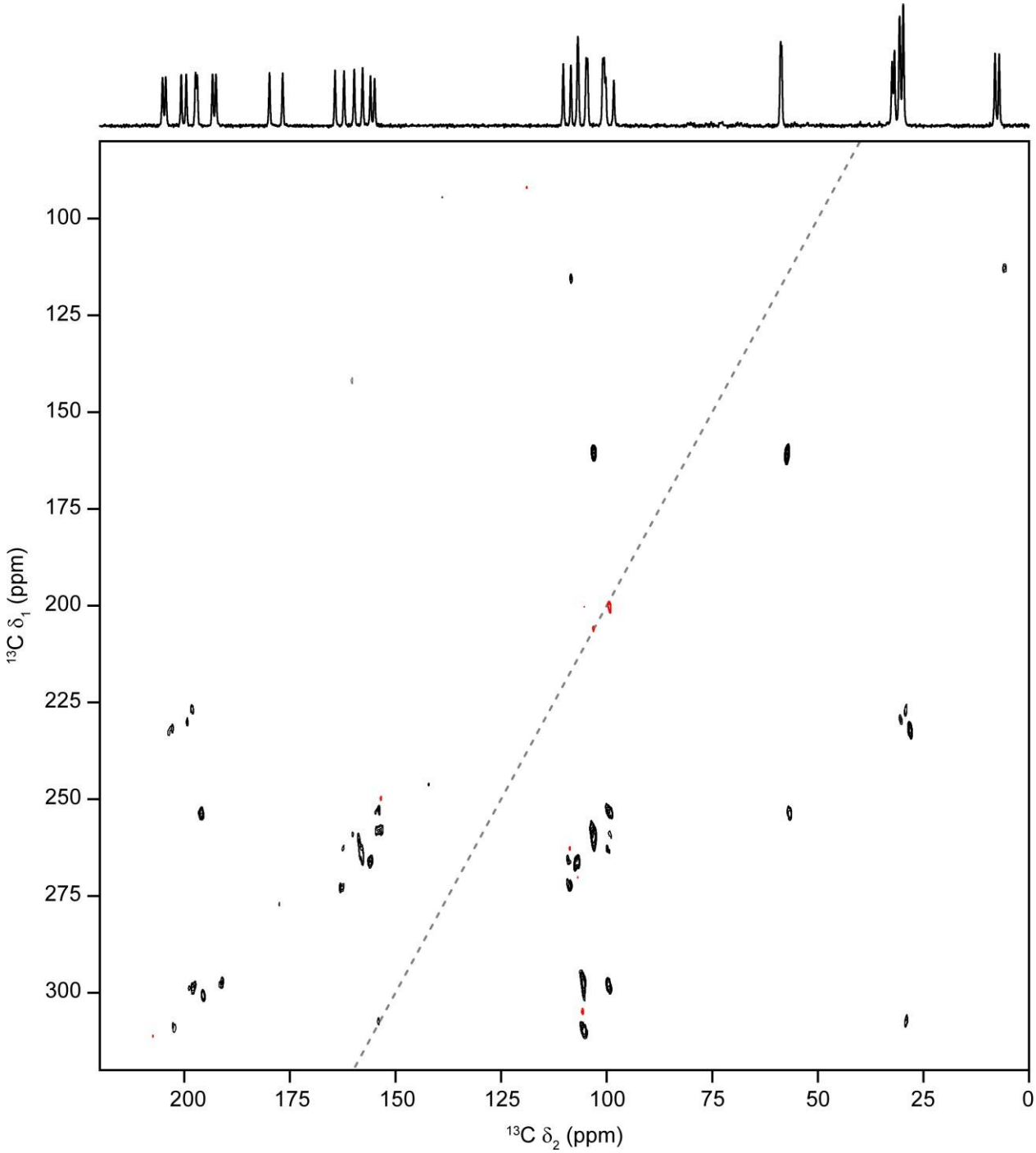

Figure S2. ^{13}C CP-INADEQUATE NMR spectrum of (+)-usnic acid (9.4 T, 12.5 kHz MAS, τ = 3.2 ms). The $\delta_1 = 2\delta_2$ autocorrelation diagonal is indicated by the dashed grey line, spinning sidebands are shown in grey, positive contours in black and negative contours in red. The ^{13}C CP MAS NMR spectrum from Figure S1b is shown along the top as a guide to the eye.

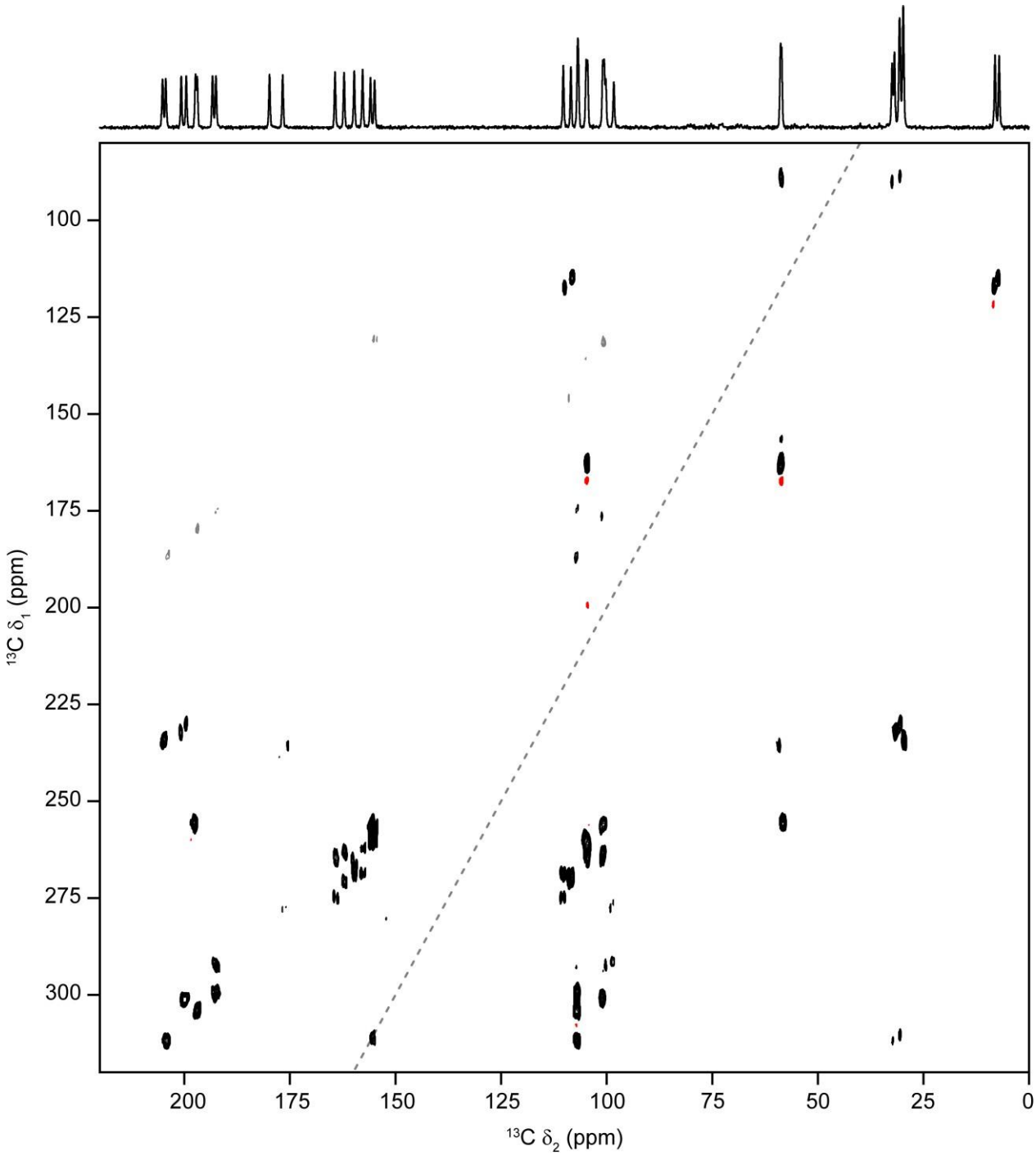

Figure S3. ^{13}C CP-INADEQUATE NMR spectrum of (+)-usnic acid (9.4 T, 12.5 kHz MAS, τ = 3.84 ms). The $\delta_1 = 2\delta_2$ autocorrelation diagonal is indicated by the grey dashed line, spinning sidebands are shown in grey, positive contours in black and negative contours in red. The ^{13}C CP MAS NMR spectrum from Figure S1b is shown along the top as a guide to the eye.

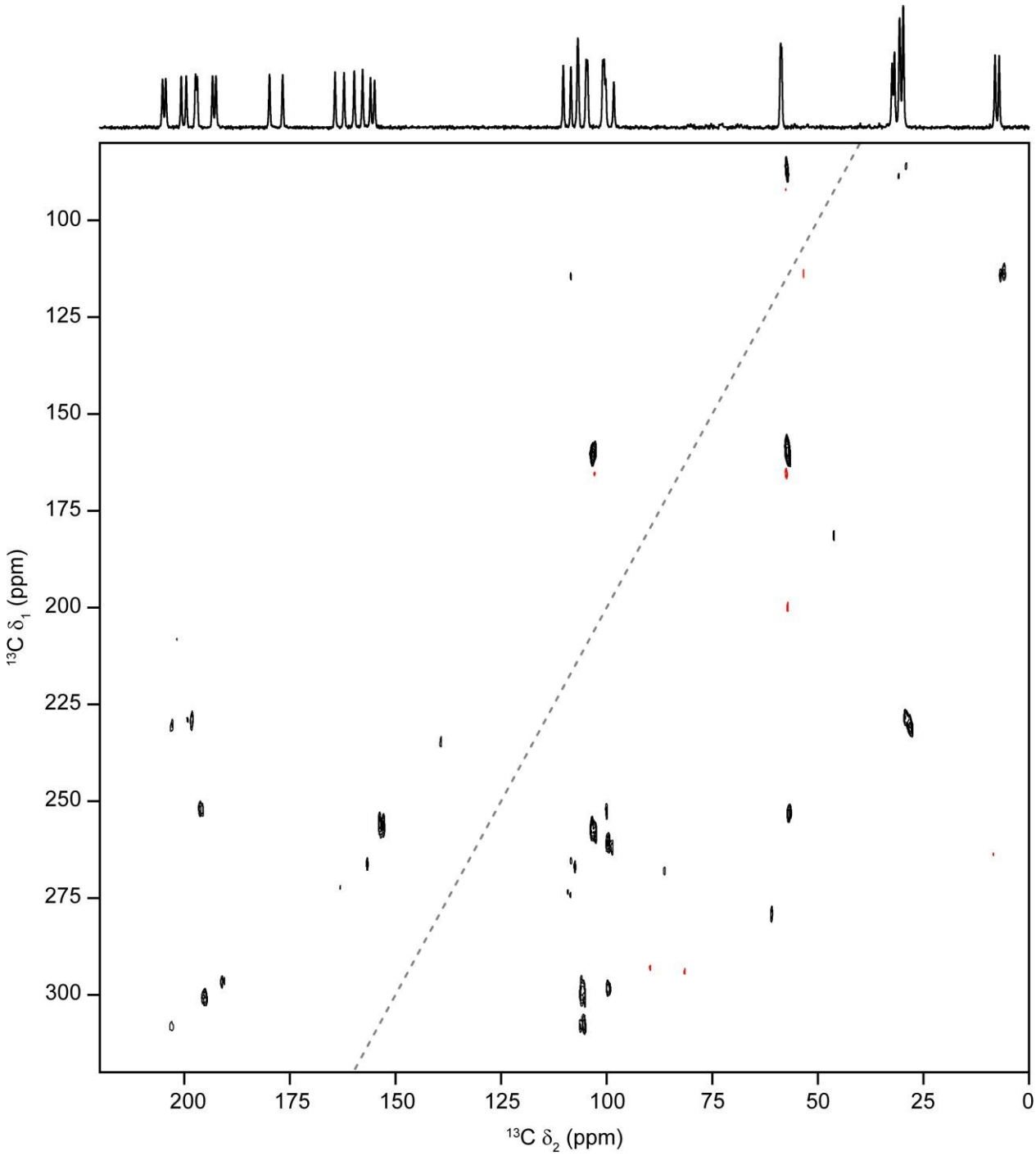

Figure S4. ^{13}C CP-INADEQUATE NMR spectrum of (+)-usnic acid (9.4 T, 12.5 kHz MAS, τ = 4.48 ms). The $\delta_1 = 2\delta_2$ autocorrelation diagonal is indicated by the grey dashed line, spinning sidebands are shown in grey, positive contours in black and negative contours in red. The ^{13}C CP MAS NMR spectrum from Figure S1b is shown along the top as a guide to the eye.

S4. ^{1}H MAS NMR Spectrum Processed with –200 Hz Weighting

Figure S5 shows the ^{1}H MAS NMR spectrum of (+)-usnic acid from Figure 6a of the main text, processed with –200 Hz exponential weighting. This processing can artificially improve the resolution, but at the expense of introducing phase and truncation artefacts. This processing allowed 12 distinct signals to be identified at 18.8, 12.4, 11.9, 11.0, 10.8, 5.5, 2.9, 2.4, 2.2, 1.8, 1.5 and 0.8 ppm.

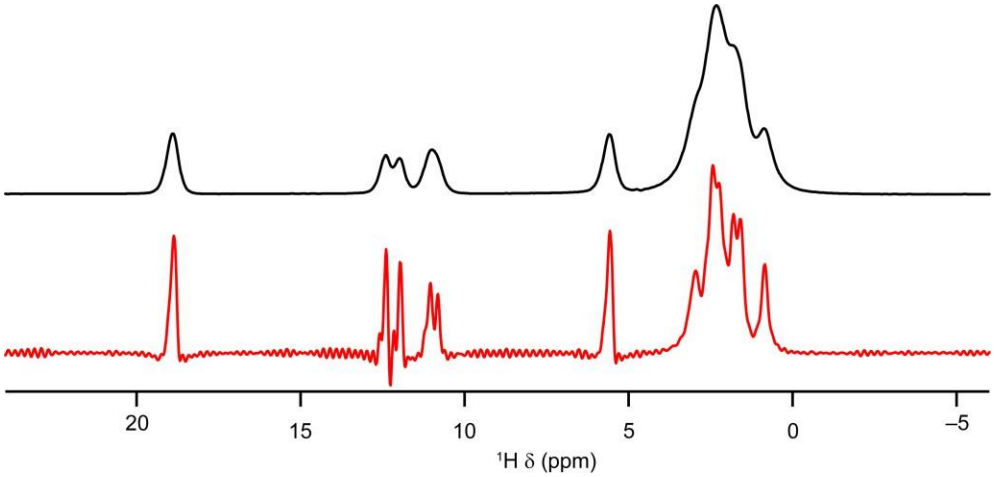

Figure S5. (a) ^{1}H (14.1 T, 60 kHz MAS) NMR spectrum of (+)-usnic acid (black, shown in Figure 6a of the main text) and the same spectrum processed with –200 Hz exponential weighting (red).

S5. Further Detail of Interpreting the NMR Spectra

Figure S6 shows the same spectrum as in Figure S3, but with coloured lines indicating groups of cross peaks that correspond to signals arising from fragments of the same crystallographically distinct molecule. These fragments are highlighted in the corresponding colours on the structures of usnic acid shown alongside the spectrum.

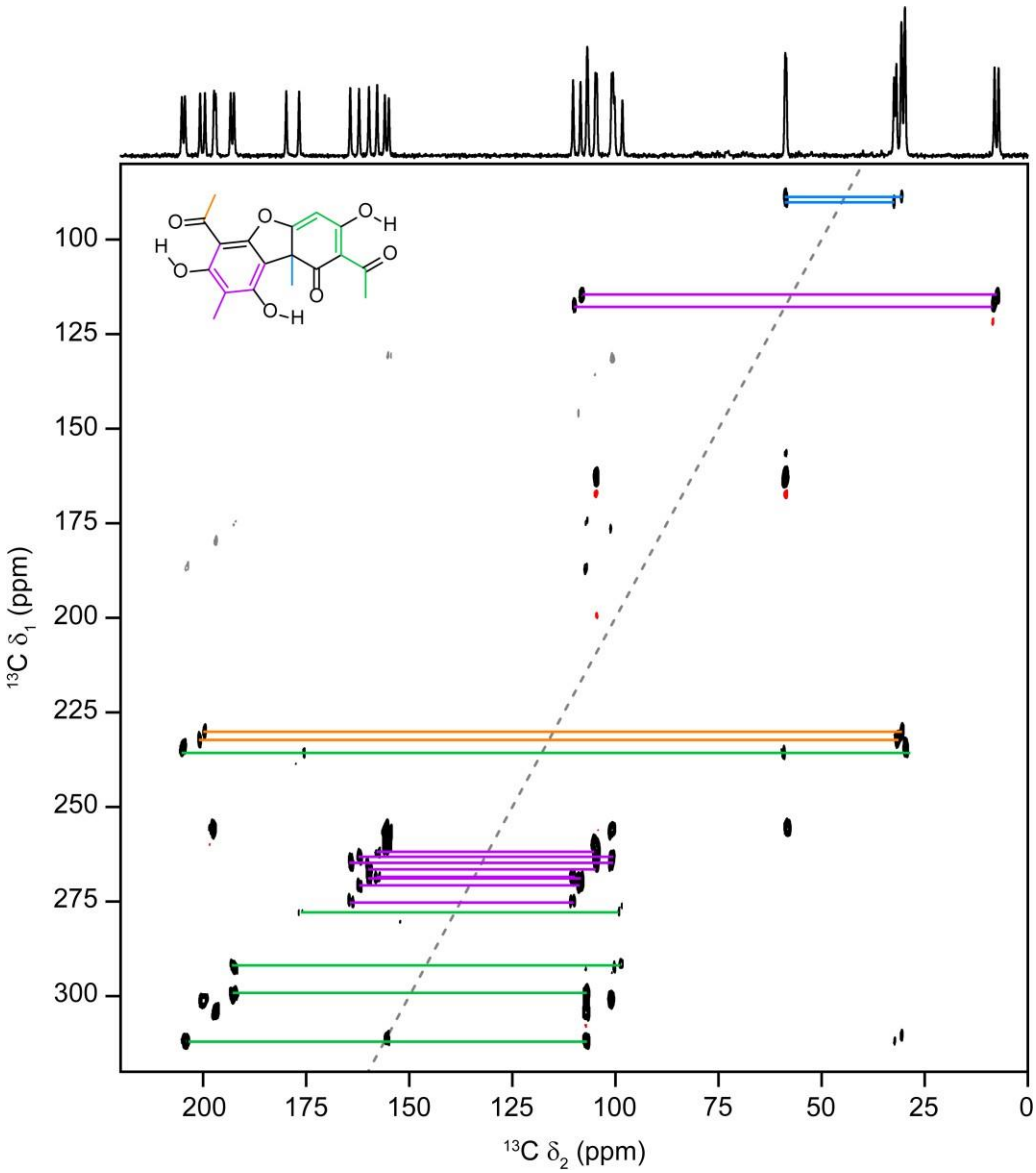

Figure S6. ^{13}C (9.4 T, 12.5 kHz MAS, τ = 3.84 ms) CP-INADEQUATE NMR spectrum of (+)-usnic acid from Figure S3, overlaid with coloured lines corresponding to the indicated molecular fragments.

The two largest fragments identified from the INADEQUATE spectrum contain carbons 4a, 4, 3, 2, 2a and B (green fragment) and 6, 7, 8, 9, 9a and C (purple fragment). Additionally, carbons 6a and

A were connected (orange fragment) as were 9b and D (blue fragment). Carbons 1 and 5a could not be connected to any fragment at this stage.

Building on the assignments shown in Figure S6, Figure S7 shows the ^{1}H-^{13}C CP-HETCOR spectrum from Figure 6c of the main text with coloured boxes indicating groups of cross peaks that allow unambiguous expansion of the two large fragments of the molecule.

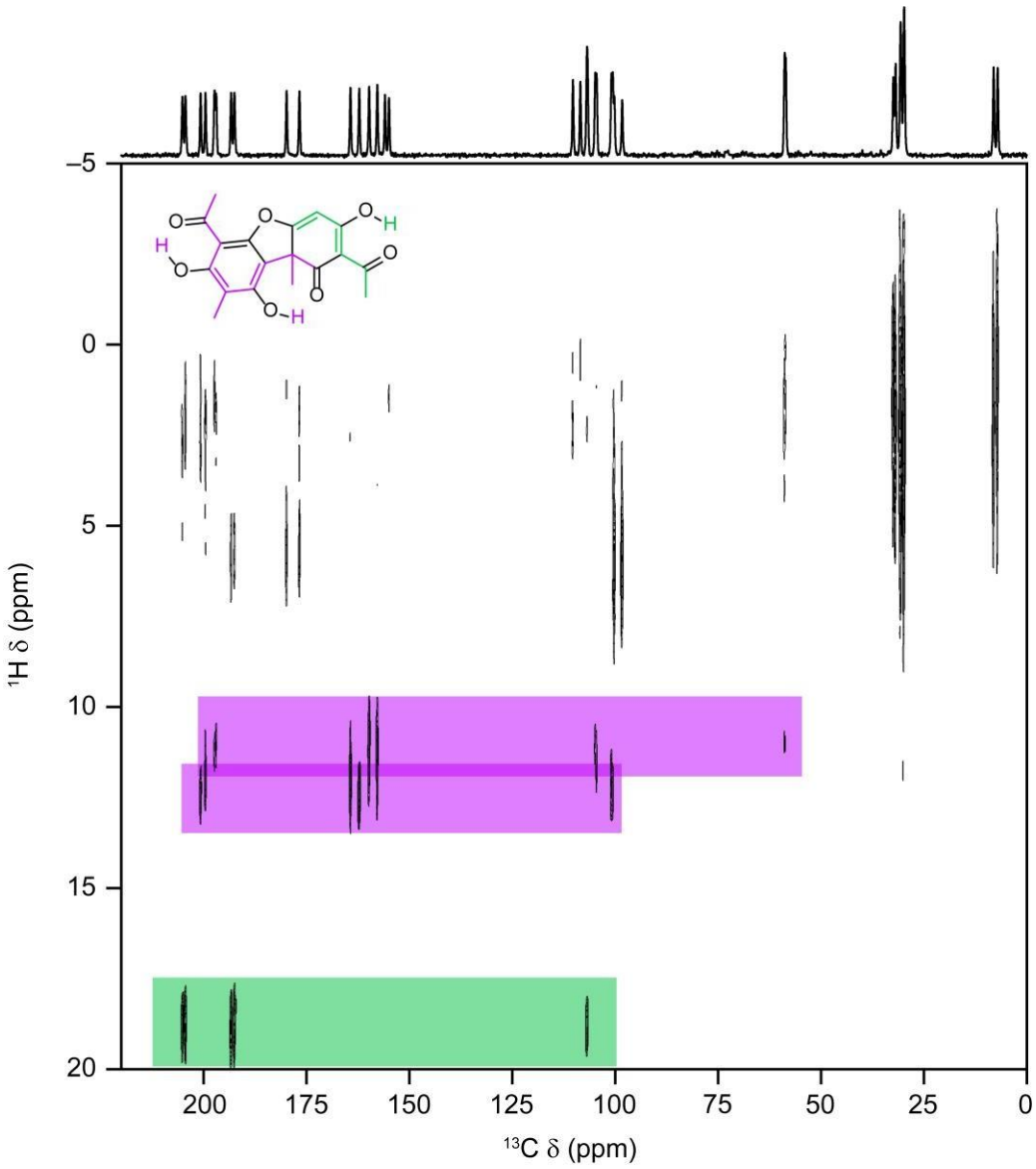

Figure S7. ^{1}H-^{13}C (9.4 T, 12.5 kHz MAS) CP-HETCOR NMR spectrum of (+)-usnic acid from Figure 6c of the main text, overlaid with coloured boxes corresponding to signals that allowed expansion and combination of the existing molecular fragments.

Following this experiment, the orange and blue fragments could be connected to the green fragment, along with carbon 1, leaving only carbon 5a not connected to either of the larger fragments.

As discussed in the main text, the ^{1}H-^{1}H SQ-DQ correlation experiment allowed the absolute assignment of the purple fragment to the corresponding crystallographic sites, as the C9(OH) proton is only spatially close to H4 on an adjacent molecule in one of the two crystallographically distinct molecules. However, as H4 has the same ^{1}H chemical shift in both molecules, it was not possible to achieve an absolute crystallographic assignment of the green fragment from experiment alone.

The green fragment contains C4a and C4, both of which have a significant chemical shift difference between their two crystallographic sites (3.1 ppm for C4a and 1.92 ppm for C4), allowing reasonably confident absolute crystallographic assignment based on the DFT calculated ^{13}C δ_{iso}. This allows complete crystallographic assignment of all ^{1}H and ^{13}C signals, with the exception of C5a. C5a has an experimental shift difference of 0.97 ppm and a calculated shift difference of 1.50 ppm with a mean absolute deviation of 0.50 ppm, meaning that it is possible to tentatively assign the signals to their crystallographic sites. These are the only signals that need to be assigned wholly computationally in the present work.

S6. Further Comparison of ^{13}C NMR Spectra of Solid (+)-Usnic Acid and *U. Dasopoga* Powder

Figure S8 shows expansions of the ^{13}C CP MAS NMR spectra of commercial (+)-usnic acid and the powdered *U. dasopoga*, shown in full in Figure 11 of the main text. It can be seen that the signals for the usnic acid align almost perfectly between the two materials, although we note a systematic shift of –0.15 ppm of all signals in the powdered lichen relative to the pure compound. As shown in Figure 9 of the main text, this cannot be attributed to different sample temperatures, and we have confirmed that the difference does not arise from a referencing error. We suggest that the magnetic influence of the lichen matrix is responsible for this effect.

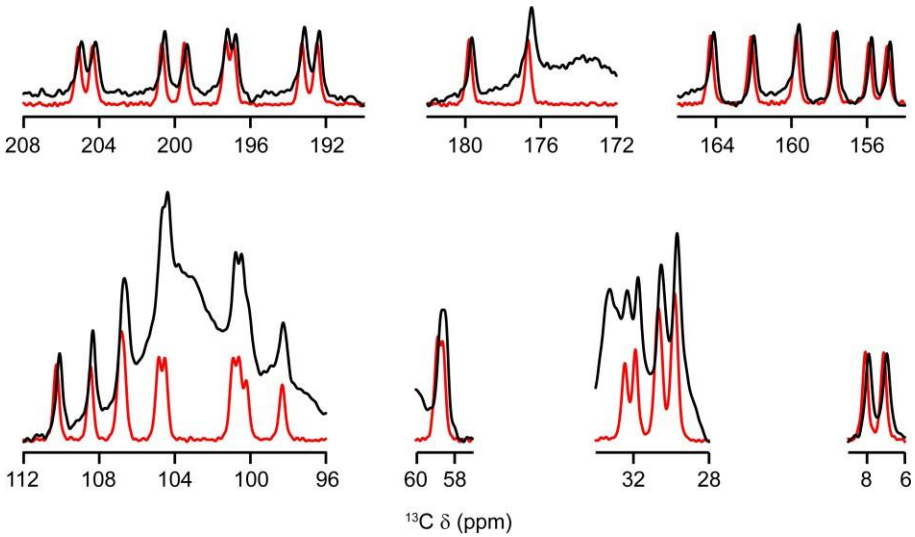

Figure S8. Expansions of the ^{13}C CP MAS NMR spectra (9.4 T, 12.5 kHz MAS) of ground lichen (black) and commercial (+)-usnic acid (red) shown in Figure 11 of the main text.

S7. References

S1. D. G. Cory, W. M. Ritchey, *J. Magn. Reson.* 1988, **80**, 128–132.

S2. A. Bax, R. Freeman, T. A. Frenkiel, *J. Am. Chem. Soc.* 1981, **103**, 2102–2104.

S3. A. Lesage, M. Bardet, L. Emsley, *J. Am. Chem. Soc.* 1999, **121**, 10987–10993.

S4. M. Feike, D. E. Demco, R. Graf, J. Gottwald, S. Hafner, H. W. Speiss, *J. Magn. Reson. A* 1996, **122**, 214–221.