Supporting Information

for

Trinorlupeol: a major non-sterol triterpenoid in *Arabidopsis*

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Contents

Materials and methods

Chemicals. Bis(trimethylsilyl)trifluoroacetamide (BSTFA), cholesterol ethyl ether, β-amyrin, and lupeol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents were Omnisolve grade from EM Science (Gibbstown, NJ, USA). $CDCl_3$ for NMR (Cambridge Isotope Laboratories, Andover, MA, USA) was filtered through basic alumina prior to use.

Plant growth conditions. Wild-type *A. thaliana* var. Columbia (Col-0) seeds were surface-sterilized in 30% bleach containing 0.01% Triton X-100 for 10 min, rinsed twice with sterile water, and grown aseptically on plant nutrient medium[1] solidified with 0.1% agar. After growth for 7-9 days at 22° C under continuous white light, the seedlings were transplanted to soil (Metro-Mix 200, Scotts, Marysville, OH, USA) and grown at 22 °C under either continuous or 8-h white light.

Liquid chromatography. AccuBond SPE cartridges (500 mg or 1 g of silica gel; J&W Scientific/Agilent) were preconditioned with hexane and eluted with CH_2Cl_2-hexane and MTBE-hexane mixtures. Reverse-phase preparative HPLC was done using an Agilent 1100 system with an Alltech Alltima 5-μm C_8 column (150 mm × 22 mm i.d.), a Phenomenex Prodigy 5-μm C_{18} column (250 mm × 21.2 mm i.d.), or a Cadenza 3-μm CD-C_{18} column (250 mm × 4.6 mm i.d.; Silvertone Sciences, Philadelphia, PA, USA) using methanol-water gradients. After preliminary monitoring of the HPLC eluate by UV detection at 210 nm, triterpenoid components were identified by GC-MS analysis of individual HPLC fractions.

Preparative TLC (PTLC). PTLC was done on 20 × 20 cm silica gel plates (250-μm layer) that had been washed by developing in 1:1 methanol-CH_2Cl_2 and activated in a 100 °C oven for ca. 18 h. Lipid mixtures were spotted onto the plate, which was developed with CH_2Cl_2. With the aid of a UV lamp, the plate was divided into several bands. Each band of silica was scraped onto a small column and eluted with MTBE, followed by spectral analysis and further chromatographic separation.

GC-MS. GC-MS data were obtained with an Agilent system comprising a 5973N MSD interfaced to a 7683 autosampler and 6890N GC containing an Rtx-35 capillary column (Restek, 30 m × 0.25 mm i.d., 0.10 μm film thickness, 35% diphenyl / 65% dimethyl polysiloxane). Samples (2 μL) of TMS ethers in the derivatization solution (or, rarely, underivatized samples in hexane[2]) were injected at 280 °C in pulsed splitless mode, with a 1-min pulse time and a pulse pressure of 1.93 bar (28 psi). Helium flow was constant at 1 mL/min. Mass spectra were obtained with electron-impact ionization at 70 eV (230 °C ion source) over a mass range of 50–650 Da. The initial GC oven temperature was held at 110 °C for 1 min, increased at 40 °C/min to 250 °C, then increased at 2 °C/min to 255 °C, held at 255 °C for 13 min, and finally increased at 5 °C/min to 280 °C. Analytes were identified by comparing GC retention times and mass spectra with those of authentic standards.

Trimethylsilyl (TMS) ether derivatives were prepared by treating samples with 60 μL of 1:1 pyridine-BSTFA at room temperature, followed by direct injection for GC-MS analysis. Occasional suboptimal batches of BSTFA reagent required derivatization at 60 °C for 1 h.

NMR. NMR experiments were done at 25 °C in $CDCl_3$ solution containing ≤5 mM

triterpenoids. Spectra were acquired on 600- and 800-MHz Varian Inova spectrometers equipped with a cold probe (^{1}H and 2D spectra) or a 500-MHz Bruker Avance DRX instrument (DEPT, ^{13}C, and 2D spectra). Chemical shifts were referenced to internal TMS at 0 ppm (^{1}H and ^{13}C for HSQC spectra) or $CDCl_3$ at 77.0 ppm (1D ^{13}C spectra). Most ^{1}H chemical shifts were determined to ±0.001 ppm accuracy, as described previously.[3] Spectra were analyzed with Bruker xwinnmr 2.6 software.

Isolation of trinorlupeol

Aerial tissues of ~100 individual plants containing stems, floral buds, siliques and cauline leaves (total 186 g) were obtained from two harvests of *A. thaliana* (Col-0) grown in soil under continuous light at 22 °C. The fresh plant material was extracted by soaking in hexane for 2 h. Residue (417 mg) from evaporation of the extracts was chromatographed in ~50-mg portions on 1-g SPE silica cartridges. The sample was eluted with 20-mL portions of 5% and 2 × 10% CH_2Cl_2 in hexane (fractions #1-3); 1%, 2%, 2 × 2.5%, 10%, and 25% MTBE (#4-9); and neat MTBE (#10). All ten fractions were analyzed by GC-MS. The triterpene-containing fractions (#4-6, total 47 mg) were further purified by normal-phase PTLC (developed with CH_2Cl_2) and reverse-phase preparative HPLC (methanol-water gradient). In addition to β-amyrin and other triterpenes, a sample of trinorlupeol was obtained as a white solid (1.3 mg). An analytical sample of trinorlupeol (0.1 mg) was furnished by HPLC (C_{18} Cadenza column, methanol-water gradient). Characterization of trinorlupeol by chromatographic mobility, GC-MS, and NMR is described in the following sections.

Chromatographic behavior of trinorlupeol vs. triterpenes and sterols

Silica gel (PTLC and SPE cartridges). In CH_2Cl_2 or gradients of MTBE in hexane, trinorlupeol eluted slightly ahead of β-amyrin and lupeol and far ahead of sitosterol and other sterols. For PTLC developed with CH_2Cl_2, R_f values were about 0.25 for phytols, 0.20 for trinorlupeol and pentacyclic triterpenes, and 0.15 for sterols. Separating trinorlupeol from triterpenes is not readily accomplished with normal-phase PTLC or SPE.

Reversed phase HPLC. A typical elution order for methanol-water systems is trinorlupeol < lupeol < β-amyrin << sterols. On the Alltima 5-μm C_8 column (150 mm × 22 mm i.d.) with a gradient of 85:15 methanol-water to 100% methanol, retention times were 21.5, 22.3, and 22.8 min for trinorlupeol, lupeol, and β-amyrin, the latter pair being incompletely resolved. On a Phenomenex Prodigy 5-μm C_{18} column (250 mm × 21.2 mm i.d.) with a gradient of 90:10 methanol-water to 100% methanol, retention times were 41, 45.5, and 53 min.

Capillary GC. The typical elution order on diphenyl-dimethyl polysiloxane columns is trinorlupeol < campesterol < sitosterol ≤ β-amyrin < lupeol. Retention times for TMS ethers of trinorlupeol, β-amyrin, and lupeol were 9.1, 10.9, and 11.8 min, respectively. Without derivatization, the retention times were 10.3, 12.5, and 13.9 min, with the internal standard of cholesteryl ethyl ether eluting at 8.5 min. The GC conditions, which were uniform throughout this work, are given in Materials and Methods (page S2).

GC-MS characterization of trinorlupeol

Major MS fragmentation patterns of trinorlupeol are similar to those described for germanicol,[4] germanicyl acetate,[5,6] olean-18-ene,[5,6] and neohop-18-ene[6] (Figure S1). The Δ18 triterpenes have three additional carbon atoms associated with ring E and thus are higher in mass by *m/z* 42 (corresponding to C_3H_6). These fragmentation patterns are evident in the EI mass spectra of trinorlupeol (Figure S2) and its TMS ether (Figure S3). The total ion chromatogram for the TMS ether of trinorlupeol (Figure S4) indicates the high purity of the sample.

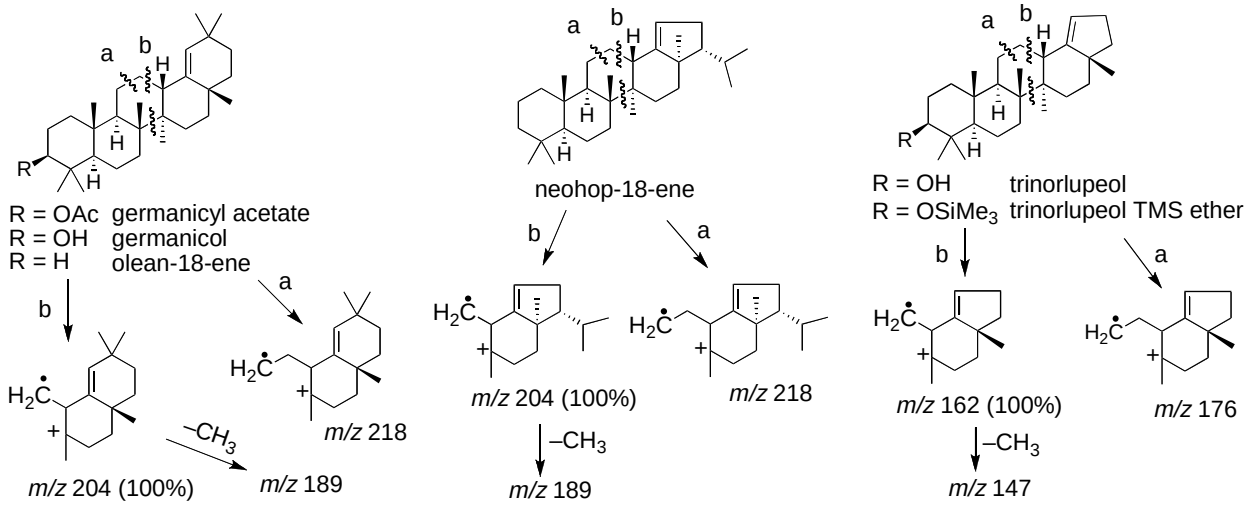


Figure S1. Conventional fragmentation mechanisms for trinorlupeol and related triterpenes. Actual mechanisms may be more complex and lead to more stable odd-electron ions.

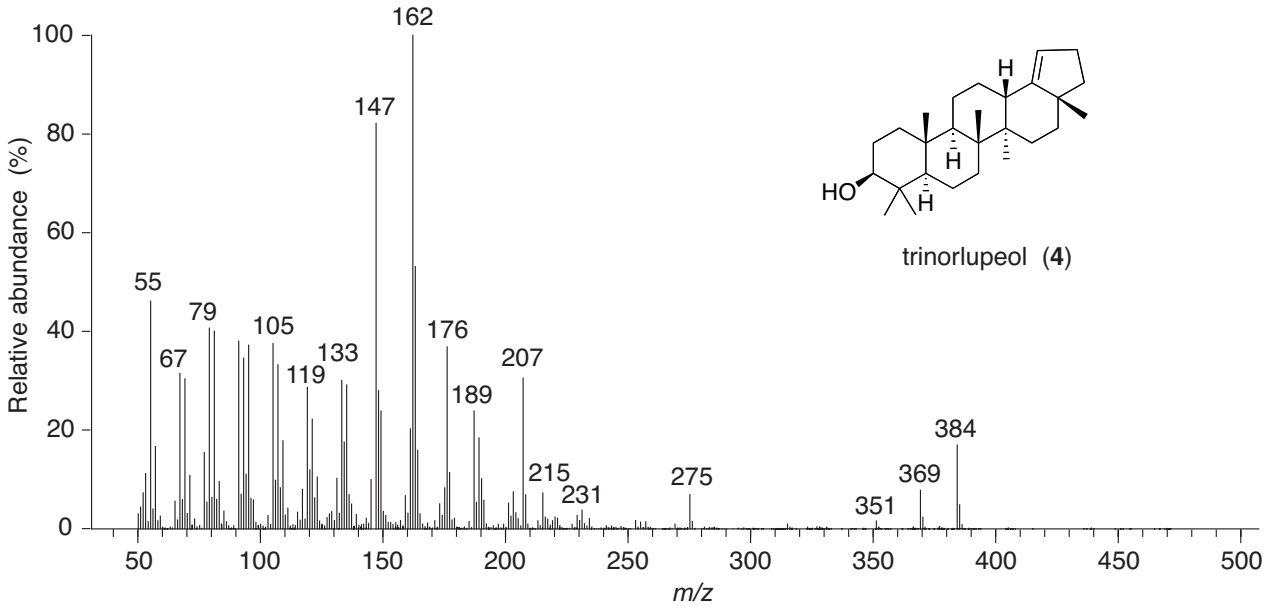


Figure S2. Mass spectrum of trinorlupeol. Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da.

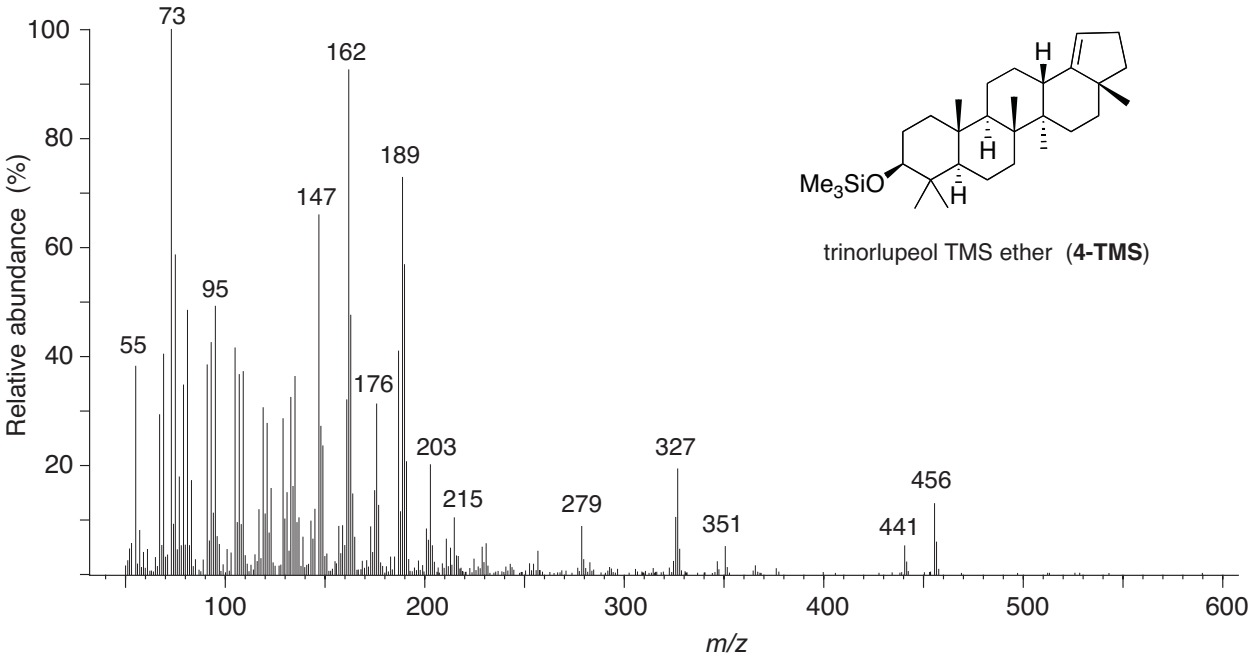


Figure S3. Mass spectrum of the TMS ether of trinorlupeol. Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da.

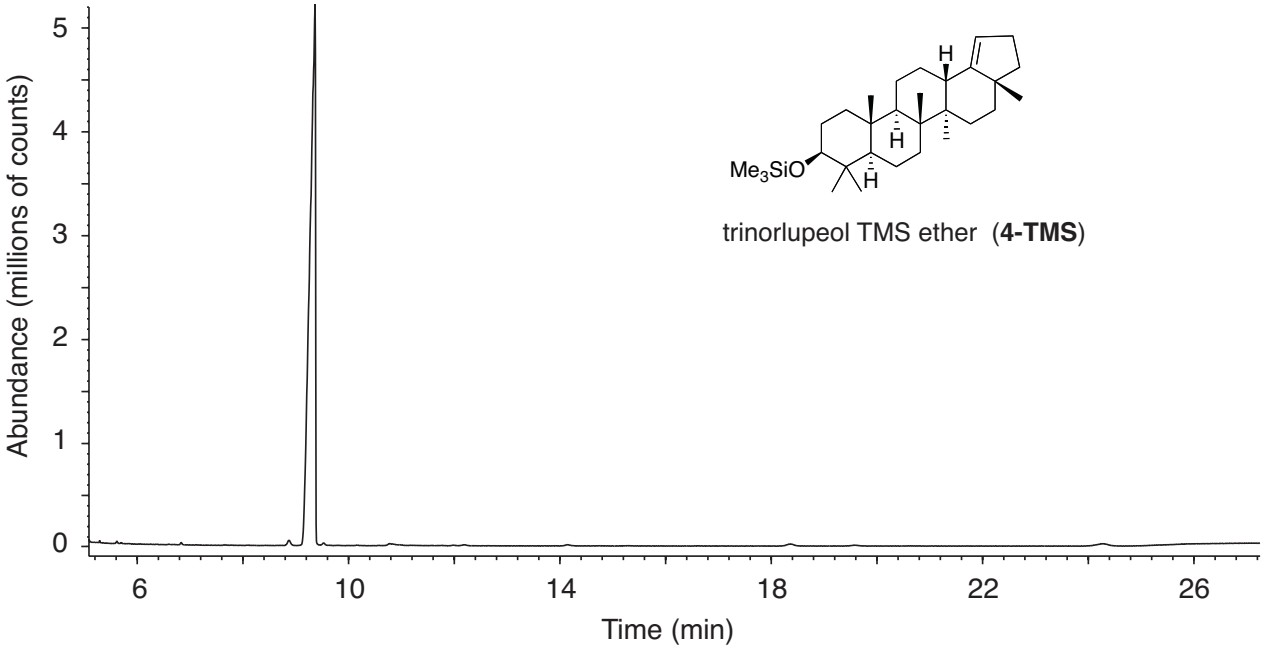
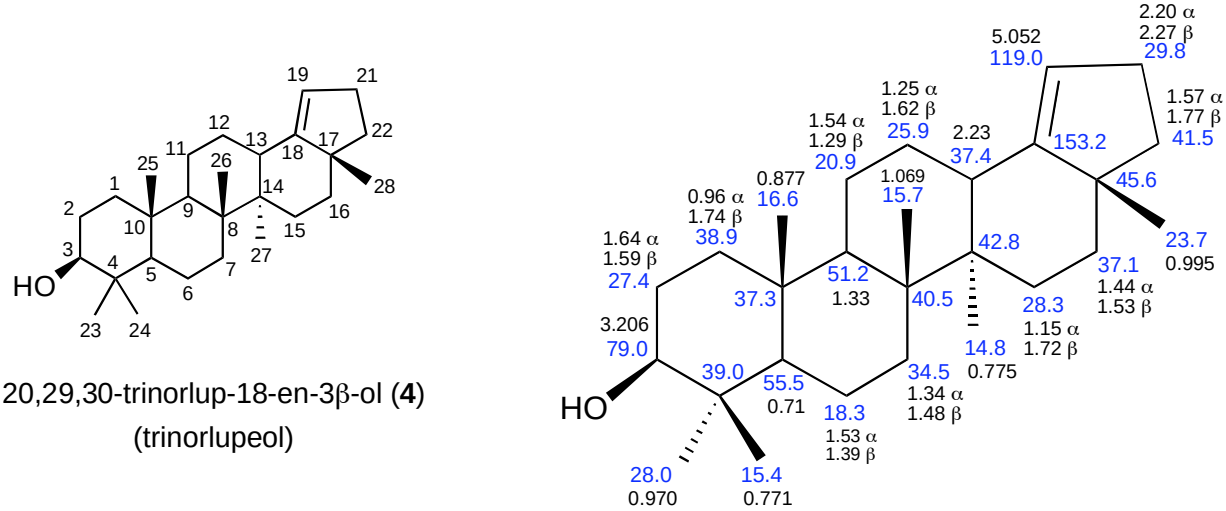


Figure S4. Total ion chromatogram of the TMS ether of trinorlupeol for *m/z* 50-650 (EI, 70 eV). The GC conditions are given in Materials and Methods (page S2). The injection was intentionally overloaded to demonstrate the purity.

1D and 2D NMR characterization of trinorlupeol

Figure S5 summarizes the atom numbering and the ^{1}H and ^{13}C NMR chemical shifts for trinorlupeol. The ^{1}H NMR spectrum is presented in Figure S6, and the ^{13}C NMR and DEPT spectra are given in Figure S7. HSQC, HMBC, and NOESY spectra are shown in Figures S8, S9, and S10, respectively. An analytical sample of trinorlupeol was used for Figure S6, whereas a sample with minor impurities was used for ^{13}C and 2D NMR spectra.



20,29,30-trinorlup-18-en-3β-ol (**4**)
(trinorlupeol)

Figure S5. ^{1}H NMR and ^{13}C NMR chemical shifts for trinorlupeol. Data are identical to those in Table 1 except for the lower precision and graphical format, which are useful for interpreting the spectra in Figures S7-S10. Conditions: 25 °C; $CDCl_3$ solution containing 1 – 5 mM trinorlupeol, referenced to internal TMS at 0 ppm (^{1}H) or 77.0 ppm (^{13}C).

Chemical shifts to ±0.0001 ppm precision can be useful for securely identifying minor sterols and triterpenes in a complex mixture when all signals except for a few upfield methyl singlets are obscured by other resonances (see Supporting Information for ref 7). Table S1 gives the methyl chemical shifts for trinorlupeol to 4 decimal places. These data were useful in the birch bark analyses described later.

Table S1. High-precision ^{1}H NMR chemical shifts for methyl signals of trinorlupeol [a]

Atom	δ_H
4α-Me	0.9704
4β-Me	0.7712
10-Me	0.8769
8-Me	1.0687
14-Me	0.7745
17-Me	0.9948

[a] NMR conditions are as described in Figure S6. The sample temperature was judged to be 24.9 °C.[8] Proton chemical shifts at ~0.5 mM (used here) and 5 mM concentration differed by up to 0.0003 ppm.

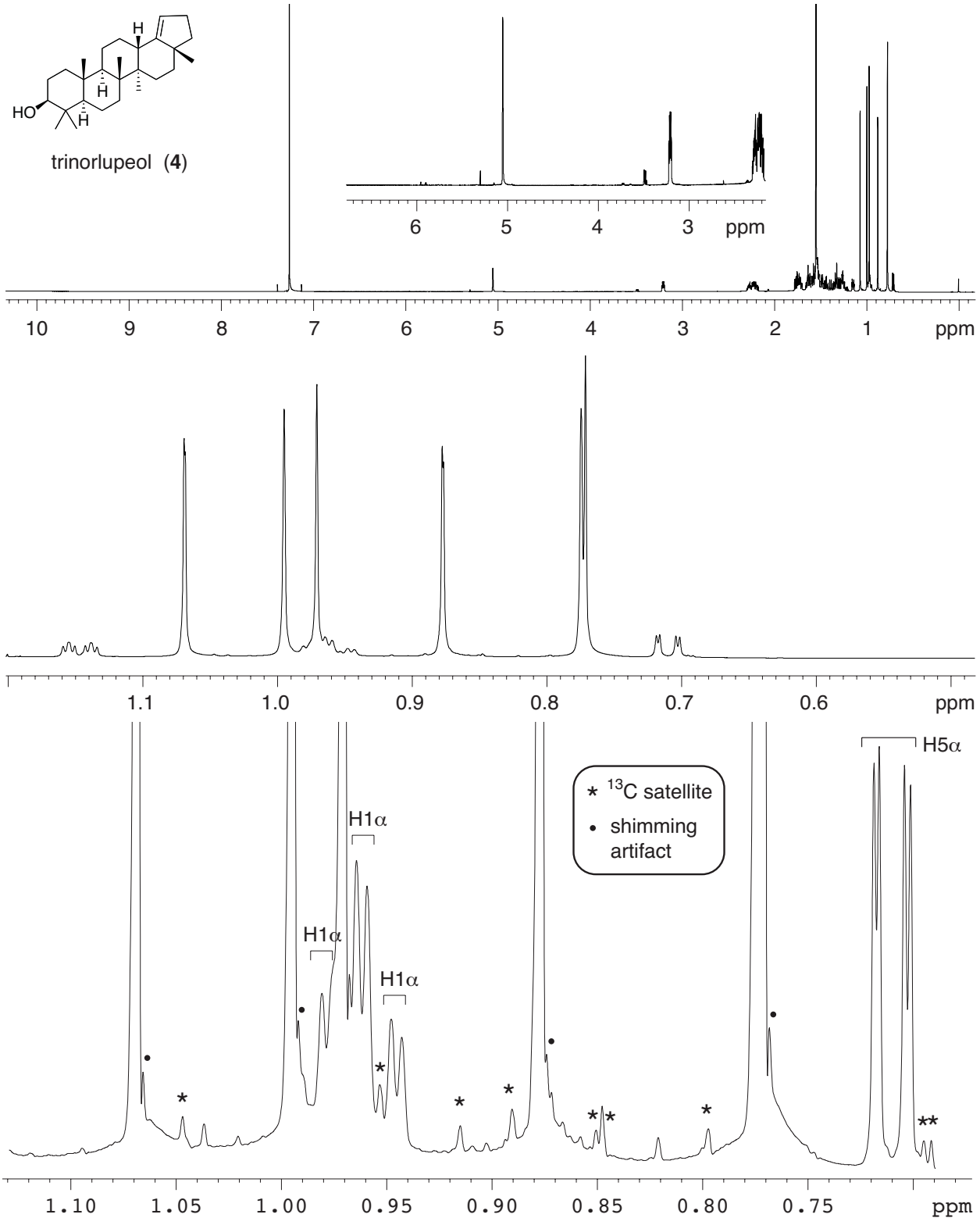


Figure S6. ^{1}H NMR spectrum of trinorlupeol: 800 MHz; 25 °C; $CDCl_3$ solution containing ~0.5 mM trinorlupeol, referenced to TMS at 0 ppm. The bottom panel establishes the absence of triterpenoid impurities above the level of 0.5%. Also absent (<0.1%) are **15**-**19**, described below.

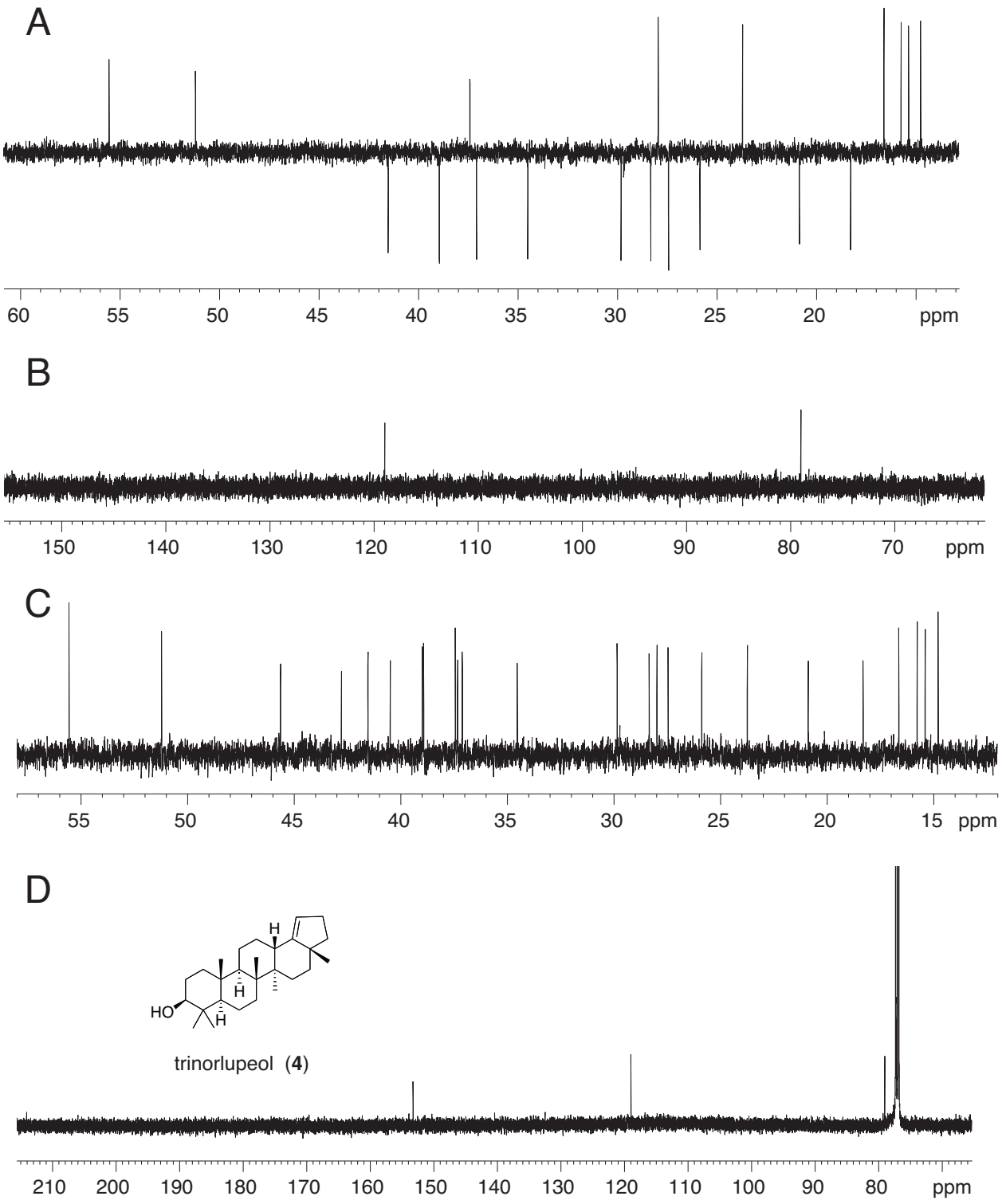


Figure S7. DEPT (panels A and B) and ^{13}C NMR spectra (panels C and D) of trinorlupeol: 125 MHz for ^{13}C; 25 °C; $CDCl_3$ solution containing 5 mM trinorlupeol. Chemical shifts were referenced to the center line of the $CDCl_3$ triplet at 77.0 ppm. Inverse-gated decoupling (1.0-s acquisition time, 3.0-s relaxation delay) mitigated sample heating in the ^{13}C NMR spectrum.

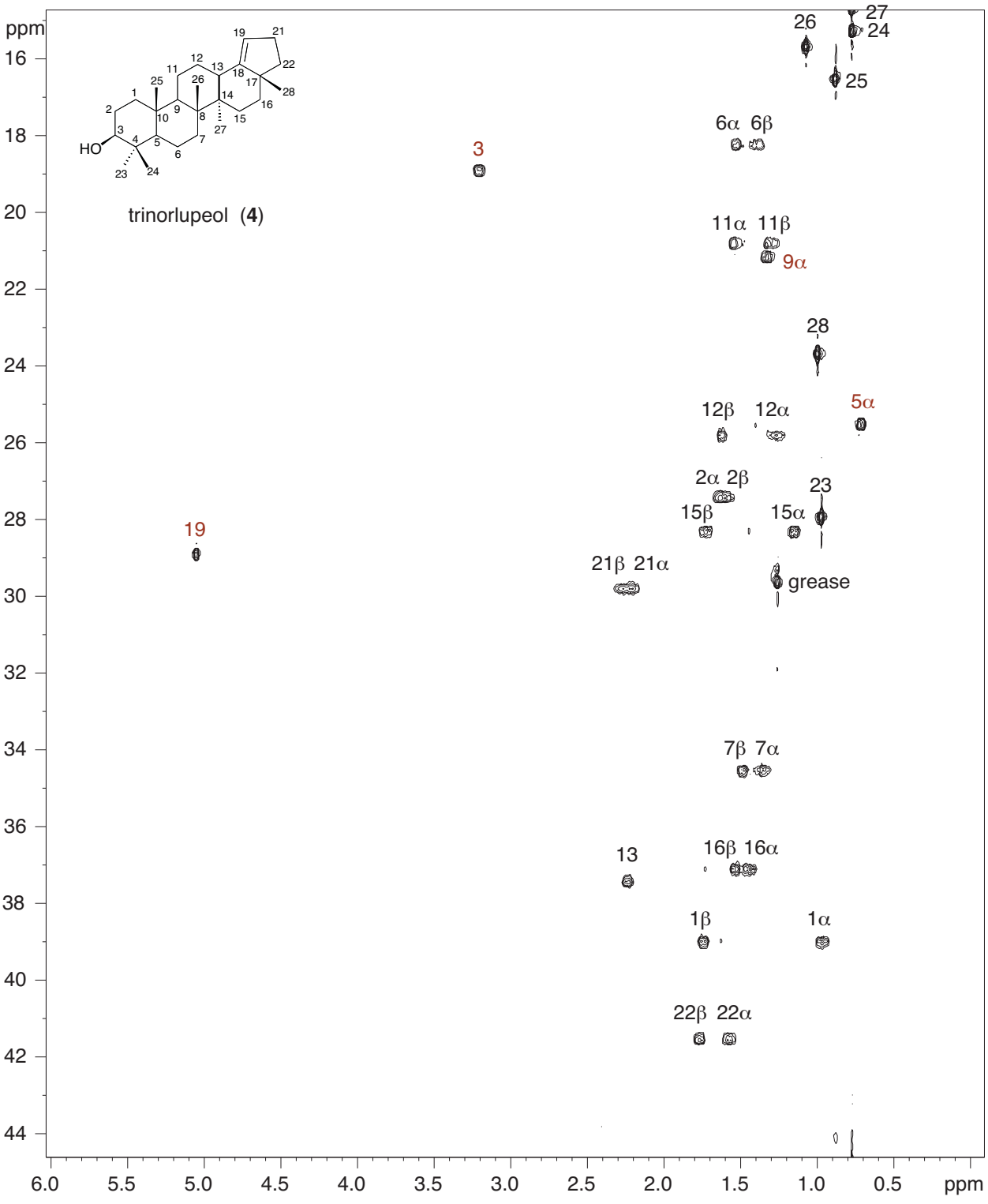


Figure S8. HSQC spectrum of trinorlupeol: 500 MHz for ^{1}H; 25 °C; $CDCl_3$ solution containing 5 mM trinorlupeol; 80 complex points in t_1; δ_C 14.4-44.4 window in f_1. Assignments for signals aliased in f_1 (by 30.0 ppm or a multiple thereof) are shown in magenta.

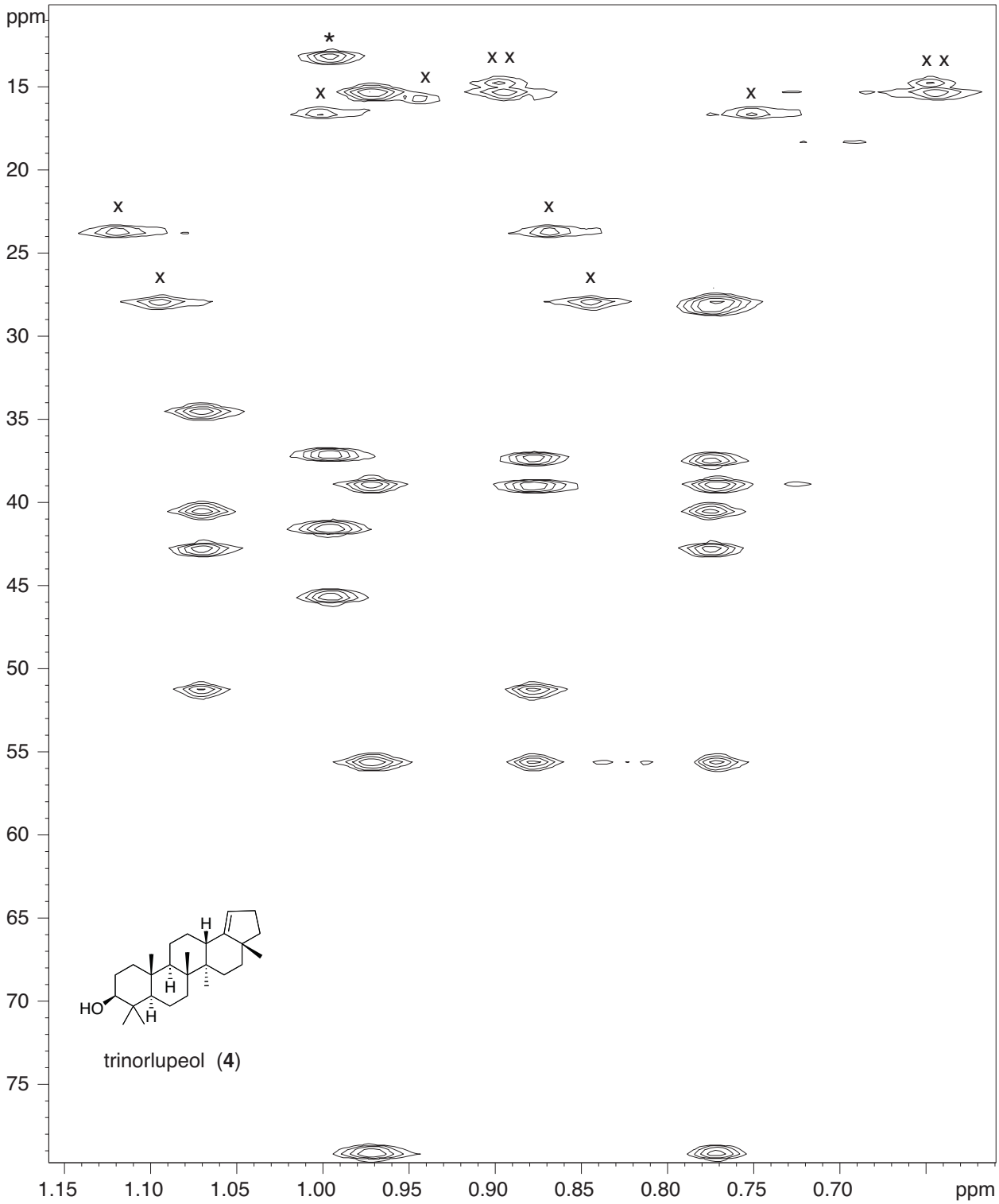


Figure S9. HMBC spectrum of trinorlupeol: 500 MHz for ^{1}H; 25 °C; $CDCl_3$ solution containing 5 mM trinorlupeol; 256 t_1 increments; δ 10-80 window in f_1. An olefinic ^{13}C correlation that was aliased in f_1 by 140 ppm is indicated by *. Artifactual signals from one-bond couplings are marked by "x".

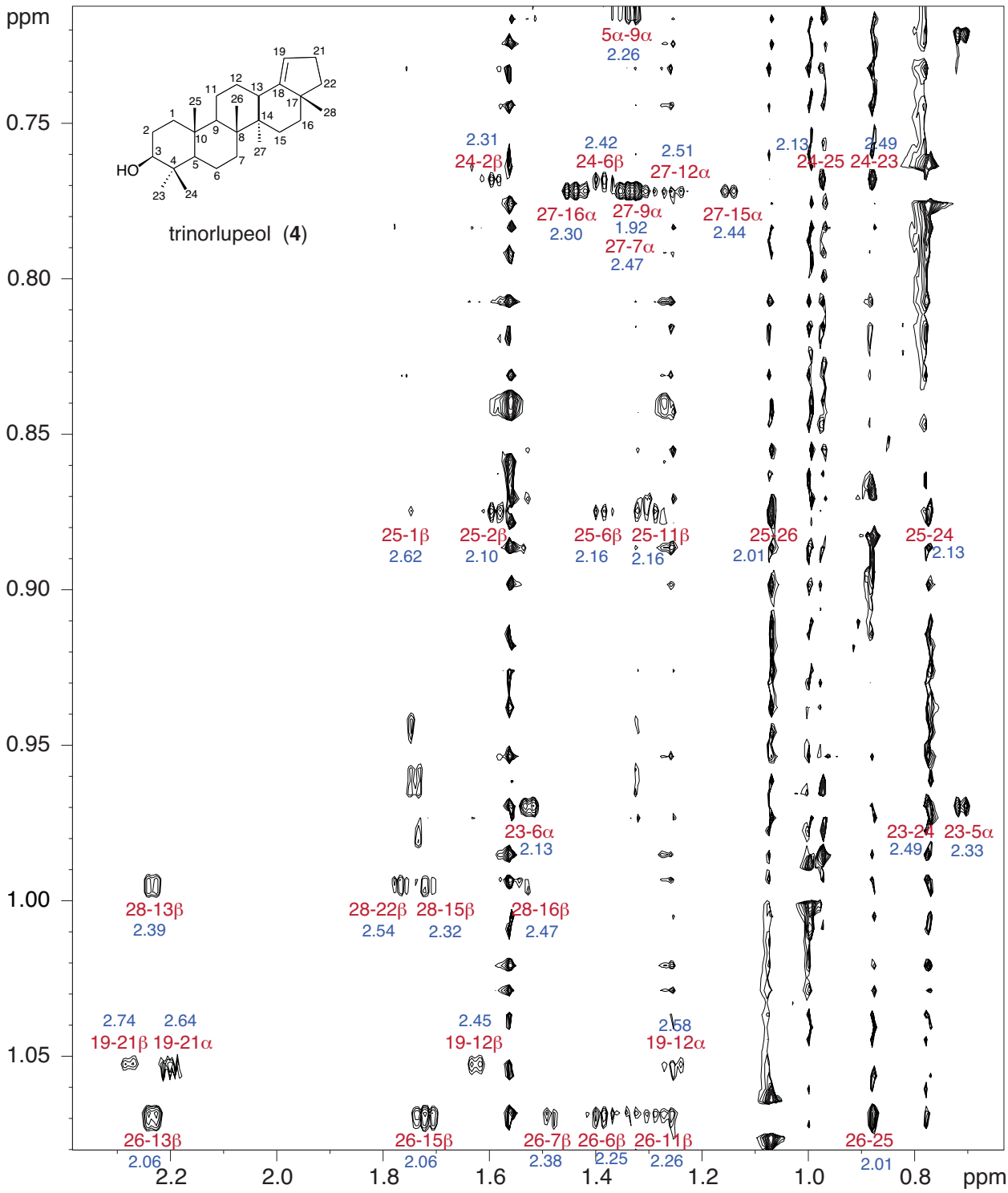


Figure S10. Expanded portion of NOESY spectrum of trinorlupeol: 800 MHz; 25 °C; $CDCl_3$ solution containing 5 mM trinorlupeol; 436 complex points in t_1; δ 8.2 to –0.8 window in f_2; δ 0.7-2.7 window in f_1; 1.5-s mixing time; 0.8-s acquisition time; 2-s relaxation delay. The olefinic signal at δ 5.054 was aliased to δ 1.054. Vertical bands of peaks represent t_1 noise. Blue numbers adjacent to signal assignments (dark red) represent interatomic distances in Å measured from a B3LYP/6-31G* structure of trinorlupeol.

Quantum mechanical calculations for NMR chemical shifts of trinorlupeol

Trinorlupeol was modeled with Gaussian 03 software.[9] The geometry of trinorlupeol was optimized at the B3LPY/6-31G* level, and NMR shieldings were calculated by the GIAO method at the B3PW91/6-311G(2d,p) level. The B3LPY/6-31G* coordinates for trinorlupeol are given below, and the NMR calculations are presented in Table S2. The rms deviations between observed and predicted values fell into the range typically found for hydrophobic compounds in $CDCl_3$ solution, i.e. 0.03-0.08 ppm for ^{1}H and 0.3-1.0 ppm for ^{13}C.


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Table S2. Observed and predicted NMR chemical shifts for trinorlupeol [a]

	^{13}C NMR chemical shifts		
atom	observed	predicted	difference
C1	38.9	38.6	-0.3
C2	27.4	28.1	0.6
C3	79.0	78.0	-1.0
C4	39.0	38.4	-0.6
C5	55.5	55.8	0.3
C6	18.3	18.6	0.3
C7	34.5	34.6	0.1
C8	40.5	41.2	0.7
C9	51.2	52.2	1.0
C10	37.3	37.2	-0.1
C11	20.9	21.3	0.4
C12	25.9	26.3	0.5
C13	37.4	37.5	0.0
C14	42.8	43.4	0.7
C15	28.3	29.7	1.3
C16	37.1	37.5	0.4
C17	45.6	45.9	0.3
C18	153.2	153.2	0.0
C19	119.0	119.9	1.0
C21	29.8	30.3	0.4
C22	41.5	41.7	0.2
C23	28.0	27.6	-0.4
C24	15.4	15.5	0.1
C25	16.6	16.9	0.3
C26	15.7	15.9	0.1
C27	14.8	15.7	0.9
C28	23.7	23.4	-0.3
	average deviation		0.26
	rms deviation [b]		0.57

	^{1}H NMR chemical shifts		
atom	observed	predicted	difference
H1α	0.96	0.95	-0.01
H1β	1.74	1.75	0.02
H2α	1.64	1.65	0.01
H2β	1.59	1.58	-0.01
H3α	3.21	3.18	-0.02
H5α	0.71	0.76	0.05
H6α	1.53	1.47	-0.05
H6β	1.39	1.37	-0.02
H7α	1.34	1.37	0.03
H7β	1.48	1.49	0.00
H9α	1.33	1.37	0.04
H11α	1.54	1.49	-0.05
H11β	1.29	1.26	-0.03
H12α	1.25	1.23	-0.01
H12β	1.62	1.61	-0.02
H13α	2.23	2.37	0.13
H15α	1.15	1.17	0.03
H15β	1.72	1.68	-0.04
H16α	1.44	1.37	-0.06
H16β	1.54	1.52	-0.02
H19	5.05	5.08	0.03
H21α	2.20	2.23	0.04
H21β	2.28	2.42	0.15
H22α	1.57	1.57	0.00
H22β	1.77	1.75	-0.02
4α-Me	0.97	0.98	0.01
4β-Me	0.77	0.72	-0.06
10-Me	0.88	0.86	-0.01
8-Me	1.07	1.06	-0.01
14-Me	0.77	0.80	0.03
17-Me	0.99	1.01	0.01
	average deviation		0.004
	rms deviation [b]		0.047

[a] Predicted chemical shifts were calculated in Gaussian 03 rev C.01 by the GIAO method at the B3PW91/6-311G(2d,p)//B3LYP/6-31G* level and adjusted with empirical corrections.[10] Differences correspond to predicted – observed values. All results were calculated at full precision before rounding to 2-4 significant figures for readability. The calculation methodology is identical to that described in our previous work except that only a single conformer was modeled herein.[11] [b] Root-mean-square deviation.

Comparison of observed and calculated ^{1}H-^{1}H couplings for trinorlupeol

^{1}H-^{1}H coupling constants for trinorlupeol were assigned from coupling patterns in Table 1 and compared with couplings calculated in PCMODEL software[12] by an extended Karplus relationship[13] using a B3LYP/6-31G* geometry. The results are shown in Table S3. The modest deviations are generally compatible with the rms deviation of <0.5 reported[10] for this calculation and thus provide a confirmation of the structure and conformation of trinorlupeol.

Table S3. Comparison of observed (Obsd) and calculated (Calcd) ^{1}H-^{1}H couplings of trinorlupeol [a]

Coupled ^{1}H atoms	Obsd J_{H-H} (Hz)	Calcd J_{H-H} (Hz)	Deviation Calcd – Obsd
Ring A			
1α-25	1.0		
1α-1β	13.1		
1α-2α	4.0	3.9	–0.1
1α-2β	13.3	13.4	0.1
1β-2α	3.4	3.0	–0.4
1β-2β	4.0	3.7	–0.3
2α-2β	13.3		
2α-3α	4.8	4.2	–0.6
2β-3α	11.8	11.2	–0.6
3α-OH	*6*		
Ring B			
5α-6α	2.2	1.9	–0.3
5α-6β	11.8	12.1	0.3
6α-6β	*12.8*		
6α-7α	*3.4*	3.5	0.1
6α-7β	*3.2*	3.4	0.2
6β-7α	*12.8*	13.3	0.5
6β-7β	2.8	3.3	0.5
7α-7β	12.3		
7α-26	0.9		
Ring C			
9α-11α	*2.6*	2.4	–0.2
9α-11β	*12.6*	12.3	–0.3
11α-11β	*12*		
11α-12α	*4*	3.7	–0.3
11α-12β	3.1	2.9	–0.2
11β-12α	*12*	13.1	1.1
11β-12β	3.6	4.0	0.4
12α-12β	12.5		
12α-13β	11.9	12.4	0.5
12β-13β	3.6 [b]	3.1	–0.5
13β-19	1.8		
13β-21α	1.7		
13β-21β	3.7 [b]		
Ring D			
15α-15β	13.2		
15α-16α	4.0	3.5	–0.5
15α-16β	2.8	3.2	0.4
15β-16α	13.9	13.3	–0.6
15β-16β	4.3	3.7	–0.6
15β-27	0.8		
16α-16β	13.4		
Ring E			
19-21α	2.6	4.5	1.9
19-21β	1.7	3.0	1.3
21α-21β	15.5		
21α-22α	9.5	9.5	0.0
21α-22β	1.7	0.4	–1.3
21β-22α	9.5	9.4	–0.1
21β-22β	7.9	9.4	1.5
22α-22β	12.4		

[a] Observed couplings were assigned on the basis of coupling patterns in Table 1 of the main text. These couplings were derived from 800 MHz ^{1}H NMR spectra and saturation difference spectra. Observed couplings have an estimated accuracy of ±0.2 Hz except for values in italics (±1 Hz). [b] We could not satisfactorily assign the 3.7-Hz coupling of the H21β resonance (ddddd, 15.5, 9.5, 7.9, 3.7, 1.7 Hz). The large H21β couplings (15.5, 9.5, 7.9 Hz) are clearly to H21α, H22α, and H22β, respectively. The H21β-H19 coupling must be 1.7 Hz because the H19 resonance (br q, 2.1 Hz) cannot accommodate a 3.7-Hz coupling. The only conceivable long-range coupling partner for the 3.7-Hz coupling is H13β. However, our analysis of H13β as ddq, 11.9, 3.6, 1.8 gives only one 3.6-Hz coupling, which was assigned to H12β-H13β. The problem could be resolved by assigning a 1.8-Hz coupling to H12β-H13β, but the H12β resonance (dq, 12.5, 3.3) does not appear to have a 1.8-Hz coupling.

Quantification of trinorlupeol from individual organs of *A. thaliana*

Tissue harvest. Fresh aerial tissues from *A. thaliana* (Col-0) were harvested from 5-month-old plants grown in soil under 8-h light/16-h dark cycles at 22 °C. "Floral buds," defined here as all tissue including stems and pedicels above the first open bud (with white petals showing), were removed with forceps. As none of the included buds were opened, the floral buds were presumed to contain no fertilized ovules. "Siliques" comprised all green siliques at least twice the size of a mature floral bud, including pedicel tissue. Siliques and cauline leaves were removed by hand picking. The remaining denuded material comprised the stems plus a small amount of pedicel tissue. The amount of organs collected (0.31 g floral buds, 2.66 g siliques, 1.11 g cauline leaves, and 6.48 g stem) exclude unneeded stems that were discarded and thus do not reflect the relative mass of each organ in the harvested plants. Harvesting was done at room temperature (ca. 23 °C) over a period of ~30 min. The material was then promptly frozen at –80 °C. Rosette leaves (2.1 g) were harvested separately from similarly grown ca. 2-month-old plants prior to the onset of flowering.

Lipid extraction. Plant tissues were extracted in two steps. First, cuticular lipids were obtained by soaking the tissue in hexanes (2 × 20 mL per gram of tissue) for 1–2 h per extraction. Then internal lipids were extracted analogously except that the solvent was 1:1 CH_2Cl_2-methanol. Within 1 h, this second extraction transferred the green color from the plant tissue to the solvent, thus indicating complete extraction of chlorophyll. This implied a thorough exposure of lipids in the membranes and cellular contents to the extraction solvent.

The pale yellow hexane extracts were directly washed with water and evaporated to a residue under a nitrogen stream. The green CH_2Cl_2-methanol extracts were evaporated under a nitrogen stream to a residue, which was dissolved in MTBE, washed with water, and evaporated to a residue under a N_2 stream. Weights of the cuticular lipid residues were typically about 0.4%, 0.3%, 0.05%, 0.2% and 0.1% of the fresh weight of the floral buds, siliques, cauline leaves, stems, and rosette leaves, respectively. Recovered internal lipids extracts were typically about 2%, 2%, 2%, 0.6% and 0.6% of the fresh weight for floral buds, siliques, cauline leaves, stems, and rosette leaves, respectively. For each crude lipid extract, triterpenoids were quantified by removing an aliquot, adding cholesteryl ethyl ether (1.2 μg), derivatizing with BSTFA, and performing GC-MS analysis.

Saponification. Methanol and KOH solution was added to each internal lipid extract to make 12.5% KOH in 75:25 MeOH-water (0.5 mL for each mg of extract). These solutions were saponified at 70 °C under nitrogen for 2 h. The samples were then allowed to cool to room temperature and extracted with MTBE (5 × 1 mL per mg of residue) and washed with water until the wash was neutral. Each organic phase was evaporated to a residue comprising the non-saponifiable internal lipids, which represented about 0.5%, 0.3%, 0.5%, 0.2% and 0.2% of the original fresh weight of floral buds, siliques, cauline leaves, stems, and rosette leaves, respectively. Aliquots were taken for quantification by GC-MS using the same procedure described for the crude extracts. The hexane extracts were not saponified.

Chromatographic purification. Cuticular lipids and the non-saponifiable internal lipids of the aerial tissues were individually purified on SPE cartridges containing 500 mg of silica gel. The samples were dissolved in ~8 mL of 6% CH_2Cl_2 in hexane and loaded onto SPE cartridges pre-conditioned with hexane. Lipids were typically eluted with 6% CH_2Cl_2 in hexane, 1%, 2%, 2.5%, 50% MTBE in hexane, and neat MTBE, respectively. One fraction (~8-10 mL) was collected for each mobile phase. All six fractions were subjected to GC-MS analysis. Typically, fraction 4 contained triterpenes, with its front or tail in fraction 3 or 5. Fraction 5, in the case of the non-saponifiable internal lipids, contained sterols (mainly sitosterol, campesterol, and dihydrobrassicasterol), with its front or tail in fraction 4 or 6.

Calibration curves and quantification. The amount of triterpenoid in each sample was determined from peak areas in the GC-MS total ion chromatogram (*m/z* 50 – 650). Prior to derivatization of triterpenoids as TMS ethers, a fixed amount (1.2 μg) of internal standard (cholesteryl ethyl ether) was added to each sample. The solution volume (60 μL) and GC injection amount (2 μL, autosampler injection) were uniform throughout this work.

Quantification was based on calibration curves (Figure S11) that compensate for the different GC-MS responses of each triterpenoid relative to the internal standard. Data for the curves was obtained by injecting 2 μL of a 1:1 BSTFA-pyridine solution (60 μL) containing the internal standard (1.2 μg) and known amounts (0.1-10 μg) of each triterpenoid, corrected for sample purity.[14] Simple linear regression analysis[15] showed a linear response (r^2 ≥0.998) over the range of triterpenoid concentrations studied (a range appropriate for the concentrations observed). TMS derivatives were used for the analysis, but the calibration is for underivatized triterpenoids.

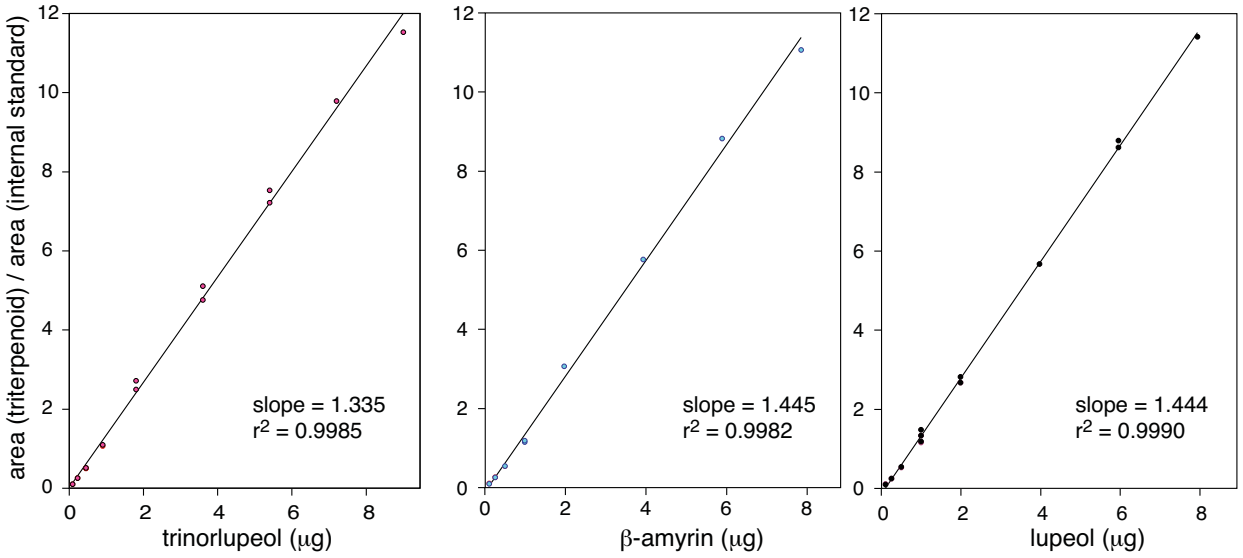

Figure S11. Calibration curves for quantification of trinorlupeol, β-amyrin, and lupeol against the internal standard cholesteryl ethyl ether. Because solution volumes were uniform in all analyses, we have for convenience plotted the mass of triterpenoid (rather than its concentration) on the abscissa. On an equimolar basis, the GC-MS response of cholesteryl ethyl ether and the TMS ethers of trinorlupeol, β-amyrin, and lupeol was 1.00, 1.37, 1.64, and 1.64, respectively.

Trinorlupeol β-epoxide and other trinorlupeol companions in *A. thaliana*

No minor compounds related to trinorlupeol were detected in the *A. thaliana* cuticular lipids except for the 3-keto derivative **5** (see below) and a trace of trinorlupeol β-epoxide **15**. In contrast, the internal lipids contained several minor compounds that may be intermediates or byproducts in the synthesis of trinorlupeol. We describe five such C_{27} triterpenoids (**15**-**19**) that were similar to trinorlupeol in chromatographic mobility on silica gel. No attempt was made to identify polar or acidic substances related to trinorlupeol.

Minor C_{27} triterpenoids from the internal lipids were isolated from material eluting on SPE immediately before the pentacyclic triterpenes. This material was combined and subjected to PTLC. The PTLC band at the upper edge of the triterpene band contained a mixture of compounds resembling trinorlupeol by NMR and/or GC-MS. This mixture was purified by preparative HPLC (Prodigy C_{18} column, methanol-water gradient). The fractions that contained trinorlupeol-like materials, as judged by NMR and GC-MS, were combined and separated by analytical HPLC (C_{18} Cadenza column, methanol-water gradient). The triterpenes observed in the analytical HPLC separation are summarized in Table S4.

Table S4. HPLC fractionation of minor *A. thaliana* triterpenoids [a]

Fractions t_R (min)	Amount (μg)	UV [b]	Triterpenoids observed by ^{1}H NMR (ratio)
14-15	~200	weak	trinorlupeol β-epoxide **15** and unknown **16** (4:1)
16-18	~20	weak	trinorlupeol β-epoxide **15**, unknown **16**, and unknown **17** (5:1:1)
21-22	~30	strong	Unknown **18** and unknown **19** (5:1)
34	~30	strong	trinorlupeol (**4**, 100%)

[a] Amounts were estimated from NMR and GC-MS sensitivity. [b] UV response at 210 nm (HPLC detector).

Trinorlupeol β-epoxide (15). Epoxide **15** was characterized by GC-MS (underivatized, Figure S12; TMS ether, Figure S13), ^{1}H NMR (Figure S14), HSQC (Figure S15), and HMBC (Figure S16). ^{1}H and ^{13}C NMR chemical shifts for **15** are summarized in Table S5. The ^{13}C chemical shifts were derived from HSQC and HMBC spectra (±0.01 and ±0.05 ppm). The ^{1}H chemical shifts were obtained from 800 MHz ^{1}H NMR spectra, together with COSYDEC and HSQC results. The epoxide configuration was deduced from quantum mechanical shielding calculations, in which the 18β,19β-epoxide predictions agreed much better with observed chemical shifts than the 18α,19α-epoxide predictions (Table S6). The combined results of GC-MS, 1D and 2D NMR, and NMR calculations established the structure of **15** as 18β,19β-epoxy-20,29,30-trinorlupan-3β-ol**.**

We initially considered that epoxide **15** might be an artifact of workup. However, **15** was also observed as a very minor component in SPE fraction 4 of cuticular lipids. This material had not been subjected to saponification or PTLC, and the mild hexane extraction and SPE conditions would not easily create epoxide artifacts from alkenes.

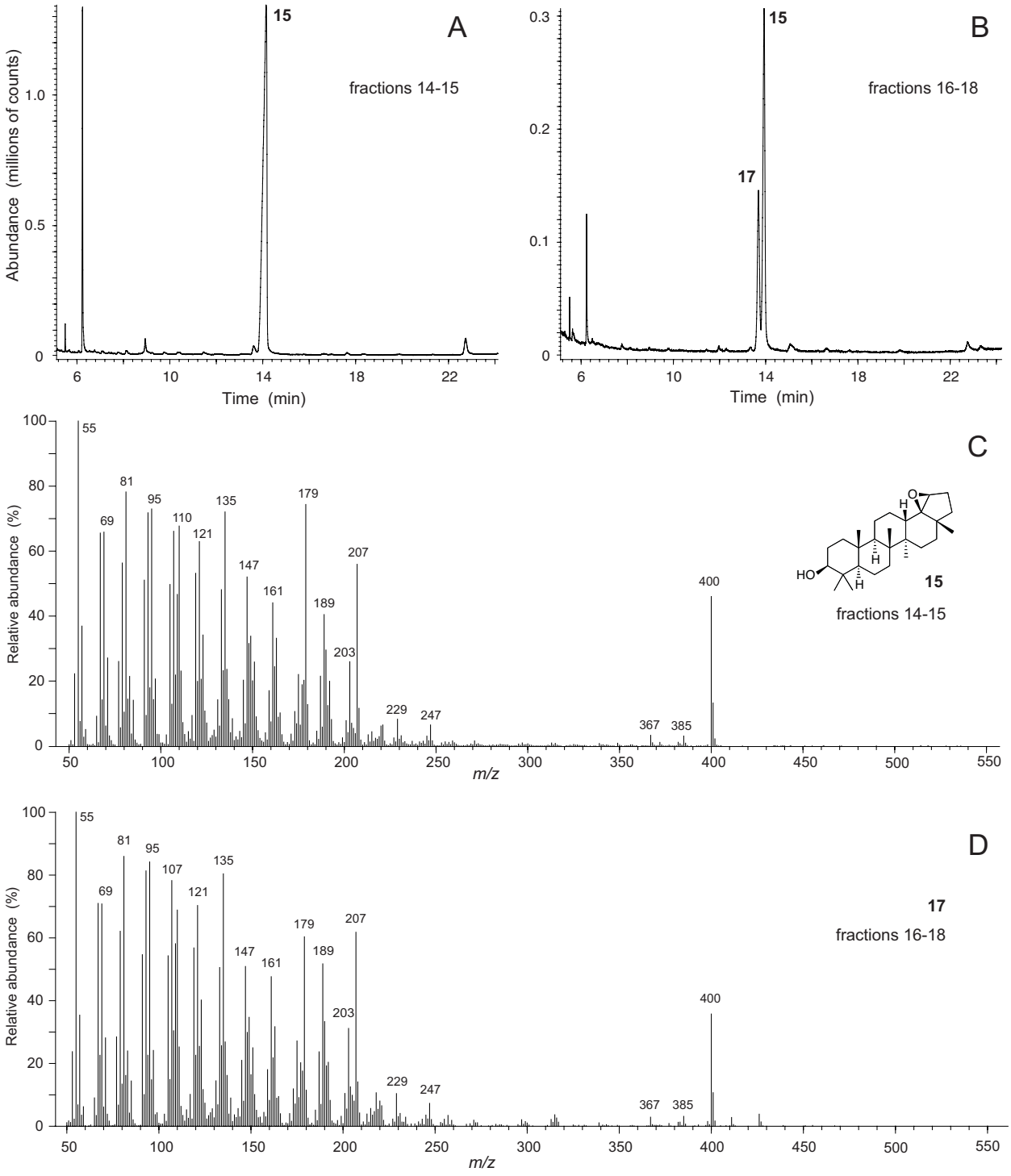

Figure S12. Total ion chromatogram (A) and mass spectrum (C) of underivatized trinorlupeol β-epoxide (**15**). Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da. For comparison are shown the total ion chromatogram (B) of adjacent HPLC fractions (16-18) and the mass spectrum (D) of a minor component (**17**) that may be the 18α,19α-epoxy isomer of **15**.

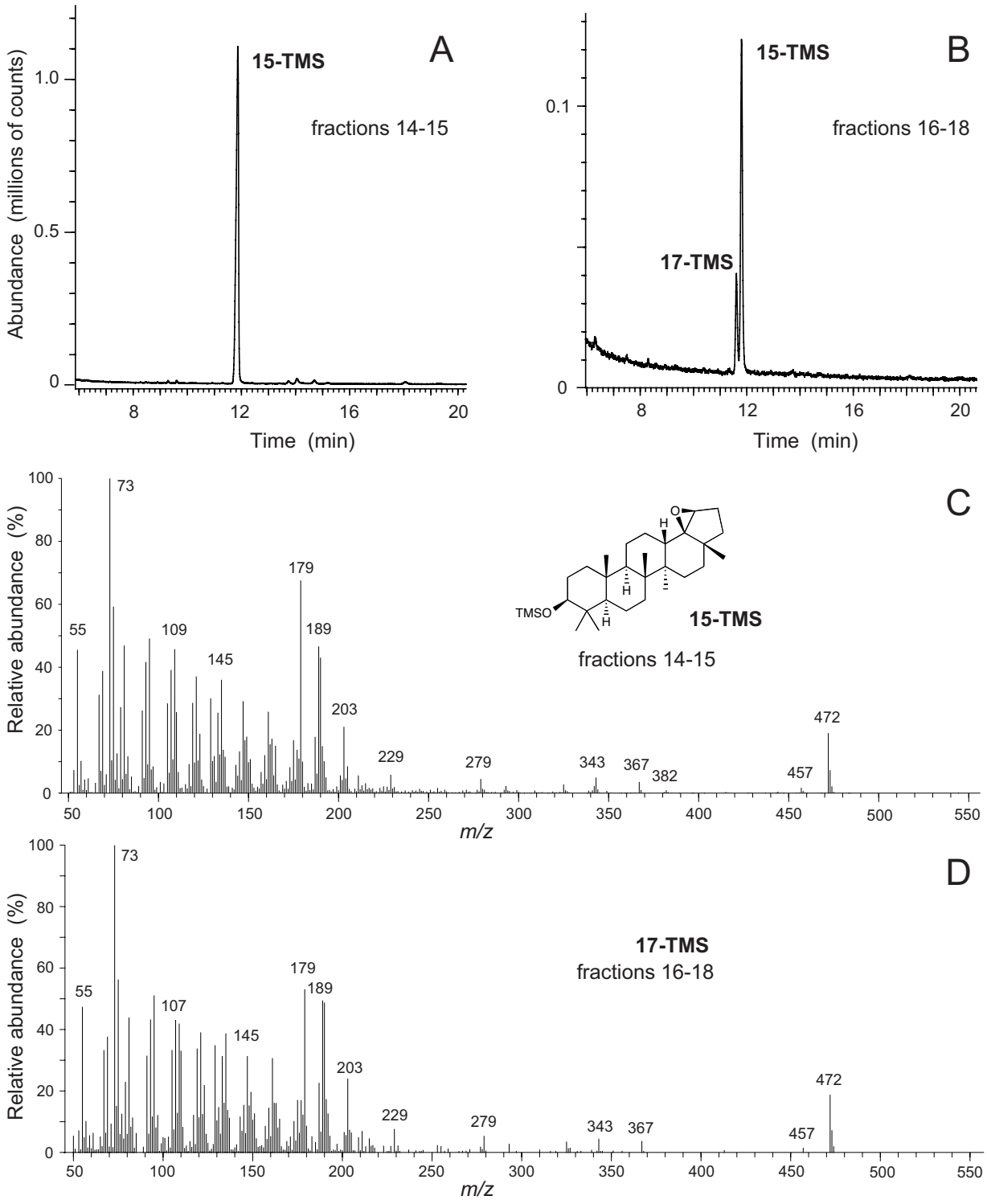

Figure S13. Total ion chromatogram (A) and mass spectrum (C) of the TMS ether of trinorlupeol β-epoxide (**15-TMS**). Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da. For comparison are shown the total ion chromatogram (B) of adjacent HPLC fractions (16-18) and the mass spectrum (D) of a minor component (**17-TMS**) that may be the 18α,19α-epoxy isomer of **15-TMS**.

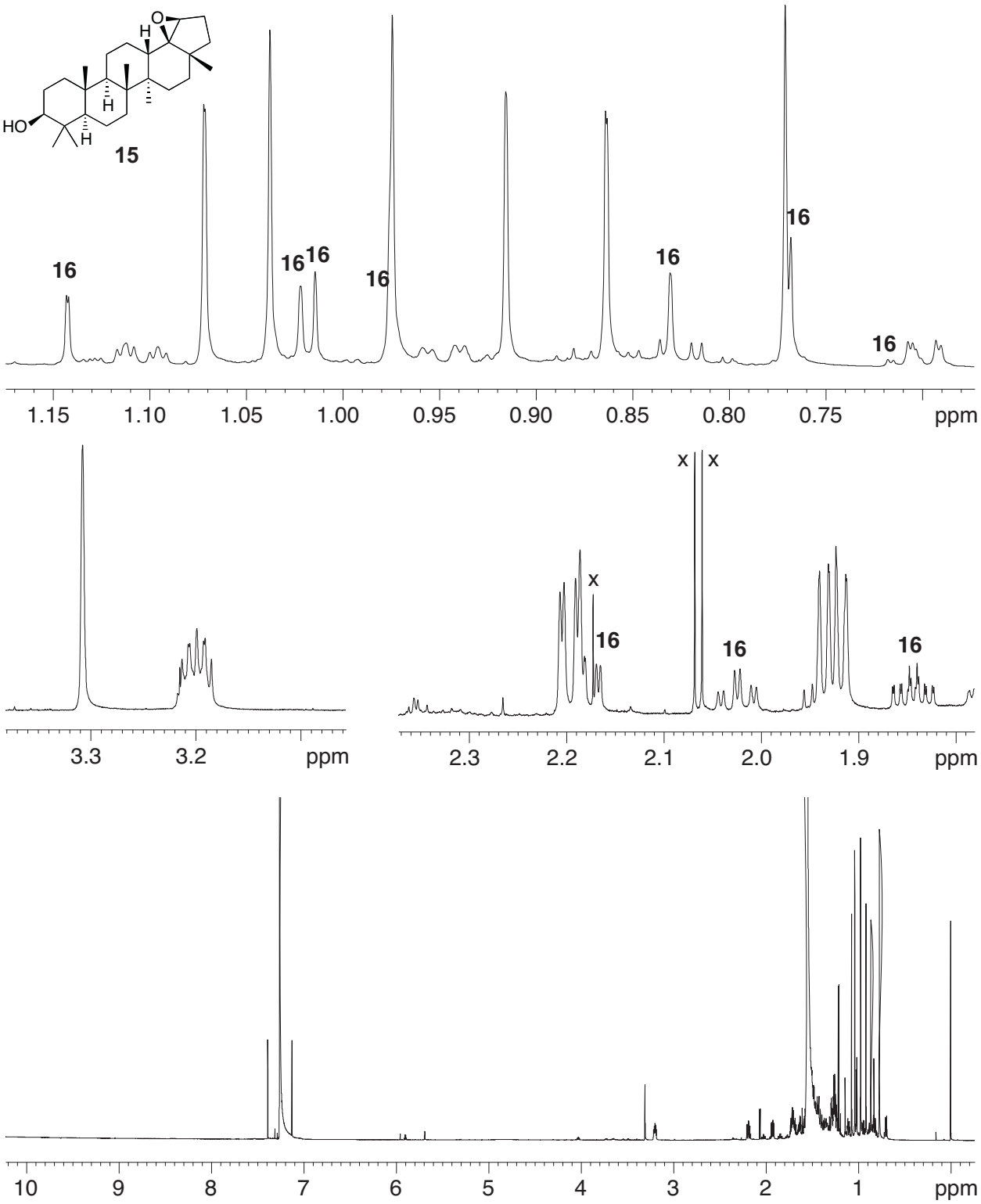

Figure S14. ^{1}H NMR spectrum of trinorlupeol β-epoxide (**15**): 800 MHz; 25 °C; $CDCl_3$ solution containing ~2 mM **15** and ~0.5 mM **16**, referenced to internal TMS at 0 ppm. Signals of the minor component (**16**) are labeled; x denotes impurities.

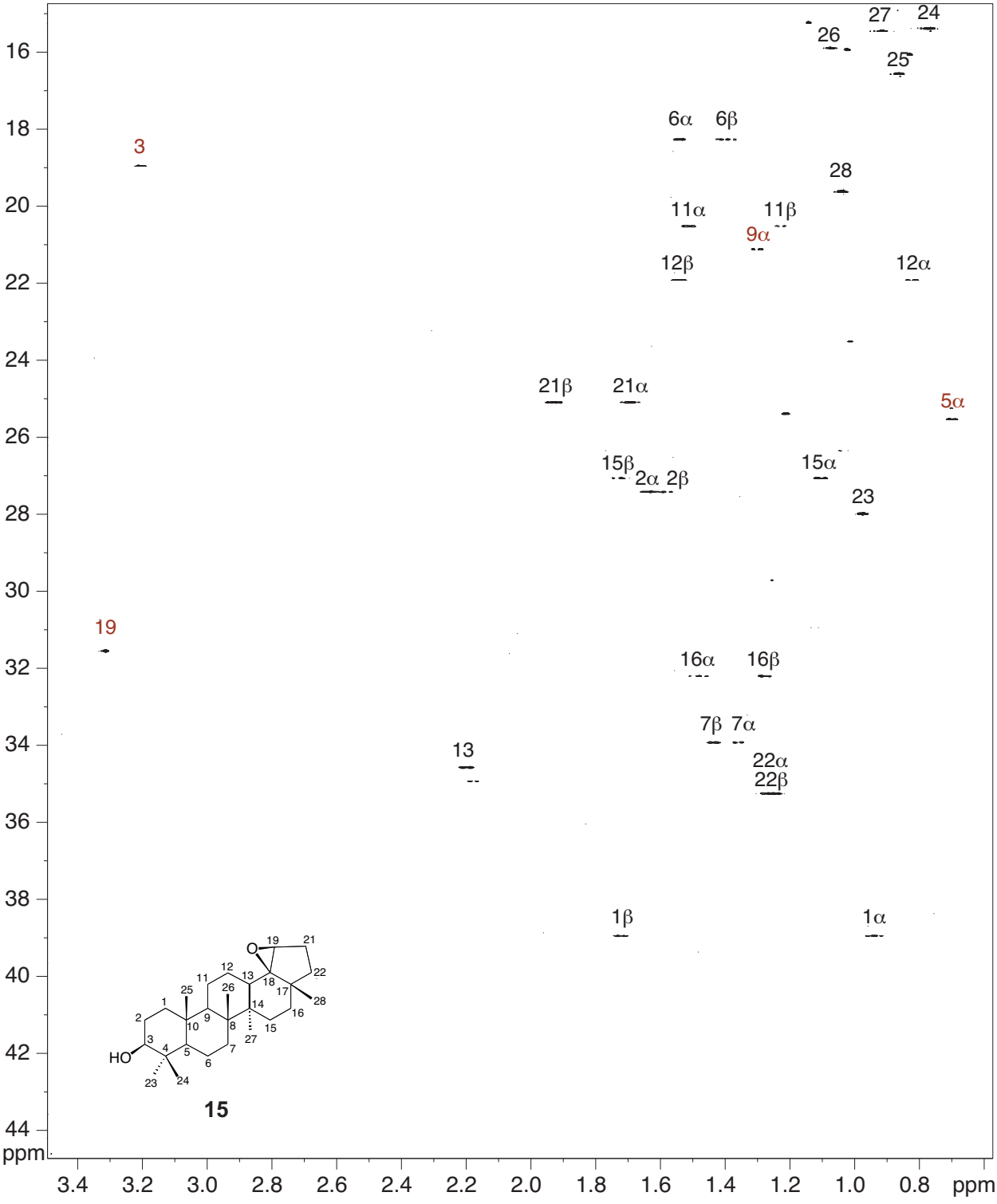

Figure S15. HSQC spectrum of trinorlupeol β-epoxide (**15**): 600 MHz for ^{1}H; 25 °C; $CDCl_3$ solution containing ~2 mM **15**; 800 complex points in t_1; δ_C 14.7-44.7 window in f_1. Assignments for signals aliased in f_1 (by 30.0 or 60.0 ppm) are shown in magenta. The sample is from HPLC fractions 14-15 and contains signals of the minor component **16**. The <6% decoupling duty cycle (0.15-s acquisition time, 2.6-s relaxation delay) minimized sample heating.

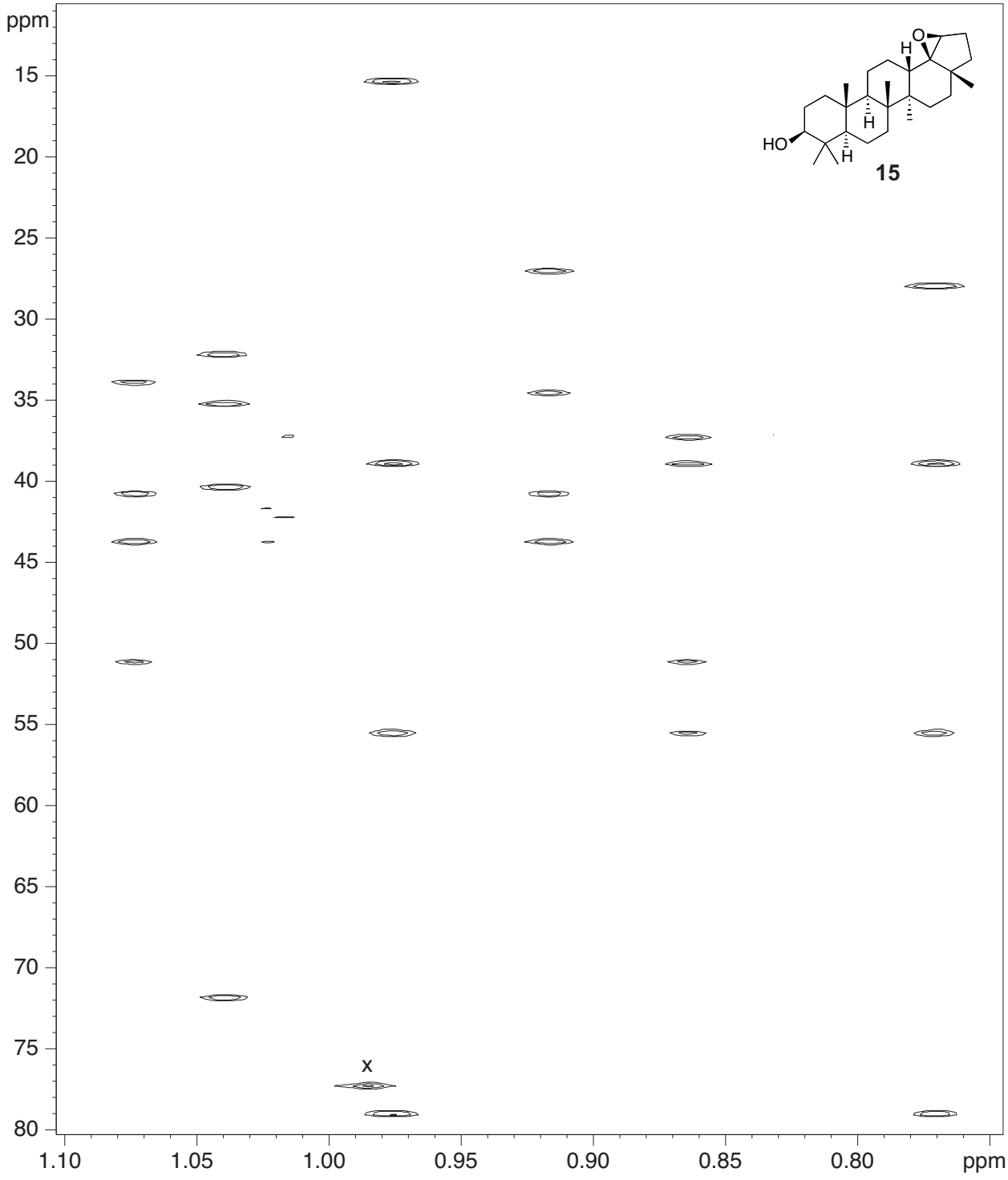

Figure S16. HMBC spectrum of trinorlupeol β-epoxide (**15**): 600 MHz for ^{1}H; 25 °C; $CDCl_3$ solution containing ~2 mM **15**; 588 t_1 increments; δ_C 10-80 window in f_1. A signal (aliased in f_2) from one-bond coupling of the $CHCl_3$ resonance is marked by "x". The sample is from HPLC fractions 14-15 and contains signals of the minor component **16**.

Table S5. ^{1}H and ^{13}C NMR data for trinorlupeol β-epoxide (**15**) [a]

^{13}C NMR			^{1}H NMR			
atom	δ_C (**15**)	$\Delta\delta_C$ [b]	atom	δ_H (**15**)	$\Delta\delta_H$ [b]	^{1}H-^{1}H couplings for **15** (Hz)
1	38.92	0.0	1α	0.939	–0.02	td, *13, 4*
2	27.40	0.0	1β	1.721	–0.02	dt, 13, 4
3	78.94	0.0	2α	1.636	–0.01	dddd, 13.5, 4.5, 4.0, 3.5
4	38.91†	–0.1	2β	1.579	–0.01	tdd, 13.3, 11.6, 3.9
5	55.52	0.0	3α	3.199	–0.01	ddd, 11.6, 6.1, 4.8
6	18.25	0.0	5α	0.699	–0.01	dd, 11.8, 2.2
7	33.91	–0.6	6α	1.541	0.02	m
8	40.71†	0.2	6β	1.400†	0.01	dddd, *14, 13, 11.5, 2*
9	51.11	–0.1	7α	1.358†	0.02	m
10	37.28†	0.0	7β	1.434	–0.05	dt, *12, 3*
11	20.51	–0.3	9α	1.300	–0.03	dd, *12.5, 3*
12	21.90	–4.0	11α	1.511	–0.03	m
13	34.55	–2.9	11β	1.228	–0.07	m
14	43.71†	1.0	12α	0.825	–0.42	qd, 13.0, 4.1
15	27.05	–1.3	12β	1.542	–0.08	m
16	32.19	–4.9	13α	2.196	–0.04	dd, 12.9, 3.6
17	40.31†	–5.3	15α	1.103	–0.04	ddd, *13.5, 4, 3*
18	71.75†	–81.5	15β	1.720	0.00	m
19	61.53	–57.4	16α	1.479	0.04	td, 13.7, 4.0
21	25.08	–4.7	16β	1.277†	–0.26	m
22	35.23	–6.3	19	3.307	–1.75	br s
23	27.97	0.0	21α	1.692	–0.51	dddd, 14.2, 10.2, 8.8, 1.3
24	15.37	0.0	21β	1.927	–0.35	ddq, 14.3, 8.1, 1.0
25	16.54	–0.1	22	1.231†	–0.34	m
26	15.88	0.1	22	1.277†	–0.49	m
27	15.44	0.7	4α-Me	0.974	0.00	s
28	19.61	–4.1	4β-Me	0.771	0.00	s
			10-Me	0.864	–0.01	d, 1.0
			8-Me	1.072	0.00	d, 0.9
			14-Me	0.915	0.14	d, 0.9
			17-Me	1.038	0.04	d, 0.5

[a] Spectra were acquired at 800 MHz (^{1}H) or 600 MHz (HSQC and HMBC spectra for ^{13}C data) at 25 °C in $CDCl_3$ solution containing ~2 mM **15** and ~0.5 mM **16**. Chemical shifts were referenced to TMS and corrected for strong coupling effects. Accuracy for ^{13}C data is about ±0.01 ppm (or ±0.05 ppm for ^{13}C values marked by †). Accuracy for ^{1}H NMR data is ±0.001 ppm (or ±0.003 ppm for ^{1}H values marked by †). Coupling constants are accurate to ca. ±0.2 Hz except for values in italics (±1 Hz). [b] The shaded values ($\Delta\delta_C$ and $\Delta\delta_H$) represent the chemical shift differences between **15** and **4**, i.e. δ(**15**) –δ(**4**).

Table S6. Comparison of observed NMR chemical shifts for trinorlupeol β-epoxide (**15**) with predicted chemical shifts for 18α,19α-epoxy-20,29,30-trinorlupan-3β-ol (α-epox) and its 18β,19β-epoxy isomer (β-epox) [a]

	^{13}C chemical shifts				^{1}H chemical shifts		
Carbon	Obsd	Deviation		Hydrogen	Obsd	Deviation	
atom	**15**	α-epox	β-epox	atom	**15**	α-epox	β-epox
C1	38.9	-0.5	-0.4	1α	0.94	-0.01	-0.03
C2	27.4	0.7	0.7	1β	1.72	-0.02	0.01
C3	78.9	-1.0	-1.0	2α	1.64	-0.01	-0.01
C4	38.9	-0.5	-0.5	2β	1.58	-0.04	-0.03
C5	55.5	0.0	0.3	3α	3.20	-0.01	-0.02
C6	18.3	0.4	0.4	5α	0.70	0.06	0.04
C7	33.9	-0.2	0.3	6α	1.54	-0.06	-0.06
C8	40.7	0.7	0.8	6β	1.40	-0.02	-0.02
C9	51.1	0.3	1.4	7α	1.36	0.12	0.03
C10	37.3	-0.2	0.0	7β	1.44	-0.05	0.01
C11	20.5	0.5	0.7	9α	1.30	0.16	0.03
C12	21.9	1.2	0.7	11α	1.51	-0.07	-0.07
C13	34.6	-1.7	0.4	11β	1.23	0.02	-0.07
C14	43.7	1.0	0.2	12α	0.83	0.53	-0.14
C15	27.1	1.2	1.3	12β	1.54	-0.51	0.09
C16	32.2	-1.0	-0.3	13α	2.20	0.29	0.14
C17	40.3	-1.2	-0.4	15α	1.10	0.02	0.00
C18	71.8	1.1	0.6	15β	1.72	0.11	-0.02
C19	61.5	-4.9	0.7	16α	1.48	0.19	-0.01
C21	25.1	0.0	-0.7	16β	1.28	0.08	-0.09
C22	35.2	-1.6	0.2	19	3.31	-0.02	0.07
4α-Me	28.0	-0.5	-0.3	21α	1.69	0.16	0.00
4β-Me	15.4	0.1	0.1	21β	1.93	-0.35	-0.04
10-Me	16.5	0.3	0.6	22α	1.23[b]	0.08	-0.15
8-Me	15.9	-0.6	-0.1	22β	1.28[b]	-0.29	0.02
14-Me	15.4	0.0	0.8	4α-Me	0.97	0.01	0.01
17-Me	19.6	1.8	-0.3	4β-Me	0.77	-0.05	-0.05
				10-Me	0.86	-0.03	-0.01
				8-Me	1.07	-0.02	0.00
				14-Me	0.92	0.21	0.01
				17-Me	1.04	-0.15	-0.06
Average deviation		-0.16	0.23	Average deviation		0.01	-0.01
rms deviation		1.27	0.62	rms deviation		0.184	0.060

[a] See footnotes for Table S2. [b] Assignments may be interchanged. Reversing these assignments has little effect on the rms deviations, which would become 0.181 and 0.066 for the α- and β-epoxides.

Spectral data for unidentified minor trinorlupeol analogs 16-19.

Unknown 16. Spectral characterization was done on a 4:1 mixture of **15** and **16** (HPLC fractions 14-15). GC-MS analyses (Figures S12 and S13) showed no minor component corresponding to 20% of the material. The possibility that **16** arose from decomposition of **15** in the $CDCl_3$ solution used for NMR analysis was excluded by reanalysis of the NMR sample by GC-MS. The total ion chromatogram and mass spectra were essentially identical to those obtained before exposure to $CDCl_3$ solution. NMR analysis of fractions 14-15 revealed many resolved signals of **16** in the 800 MHz 1D spectrum and in HSQC and HMBC spectra. These signals led to the assignments and partial structure shown in Figure S17. The structure was assembled from HMBC connectivities, as was done in Figure 2 of the main text. GC-MS, 2D NMR results, and NMR predictions (Table S6) suggested the 18α,19α-epoxide isomer of **15**, but the HMBC signal linking H28 with a downfield carbon at δ 112 excluded this possibility.

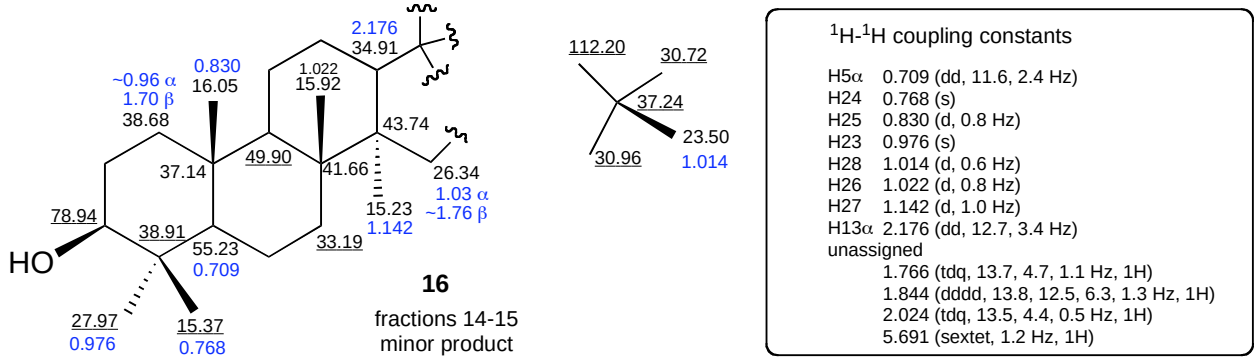

Figure S17. Partial structure and ^{1}H and ^{13}C NMR assignments for **16**, from spectra of HPLC fractions 14-15. The accuracy of the ^{13}C values was about ±0.02 ppm (from the HSQC spectrum) or, for underlined values, ±0.07 ppm (from the HMBC spectrum).

Unknown 17. Spectral characterization was done on a 5:1:1 mixture of **15**, **16**, and **17** (HPLC fractions 16-18). The GC retention time of **17** was slightly shorter than that of **15** (13.7 min vs. 13.9 min), and the same trend held for the TMS ethers (11.6 min vs. 11.8 min). However, the mass spectra of **15** and **17** were virtually identical (Figure S12), as were spectra of their TMS ethers (Figure S13). Partial ^{1}H NMR: δ_H 3.210 (br s), 3.20 (m), 1.067 (d, 1.0 Hz, 3H), 1.062 (d, 0.8 Hz, 3H), 0.973 (s, H23), 0.938 (d, ~1 Hz, 3H), 0.853 (d, 0.9 Hz, H25), 0.769 (s, H24). These NMR chemical shifts are in reasonable agreement with predicted values for the α-epoxide (Table S6), especially when the prediction errors for the β-epoxide **15** are taken into consideration. This tentative conclusion, together with the GC-MS results, suggests the 18α,19α-epoxide isomer of **15** as a candidate structure for **17**.

Unknown 18. Spectral characterization was done on a 5:1 mixture of **18** and **19** (HPLC fractions 21-22). The GC-MS retention time (8.9 min) and mass spectrum (Figure S18) were essentially identical to those of trinorlupeol (TMS ethers). Partial ^{1}H NMR: δ_H 4.940 (td, 2.0, 0.6 Hz, 1H), 4.906 (t, 2.4 Hz, 1H), 3.211 (ddd, 11, 6, 5 Hz, H-3α), 2.651 (dddt, 16.8, 10.6,

8.5, 2.4 Hz, 1H), 2.453 (br ddq, 17.0, 9.6, 1.8 Hz, 1H), 2.263 (dd, 13.1, 3.2 Hz, 1H), 2.140 (dddd, 13.8, 10.3, 9.6, 4.1 Hz, 1H), 1.930 (ddt, 13.8, 8.6, 1.3 Hz, 1H), 1.834 (br dq, 13, 3 Hz), 1.723 (dt, 13.1, 3.7 Hz, H1β), 1.134 (ddd, 12.3, 7.7, 2.9 Hz, 1H), 1.047 (d, ~1 Hz, 3H), 1.045 (d, ~1 Hz, 3H), 0.984 (s, H23), 0.863 (d, 0.9 Hz, H25), 0.780 (s, 3H, H24), 0.747 (dd, 12.0, 2.0 Hz, H5α). The GC-MS data indicate that **18** is an isomer of trinorlupeol. The limited ^{1}H NMR results suggest a rearranged trinorlupeol, in which C27 or C28 has become olefinic ($C=CH_2$ moiety). Because neither geminal olefinic proton shows long-range couplings to methyl (i.e. a quartet pattern), this is not a lupeol-type side chain ($CH_3–C=CH_2$).

Unknown 19. Spectral characterization was done on a 5:1 mixture of **18** and **19** (HPLC fractions 21-22). Partial ^{1}H NMR: δ_H 1.100 (d, 0.9 Hz, 3H), 1.042 (d, 0.6 Hz, 3H), 0.978 (s, H23), 0.832 (d, 0.9 Hz, H25), 0.768 (s, H24). The limited NMR data and lack of GC-MS results preclude any structural conclusions except that rings A and B in **19** appear to be identical to those of lupeol.

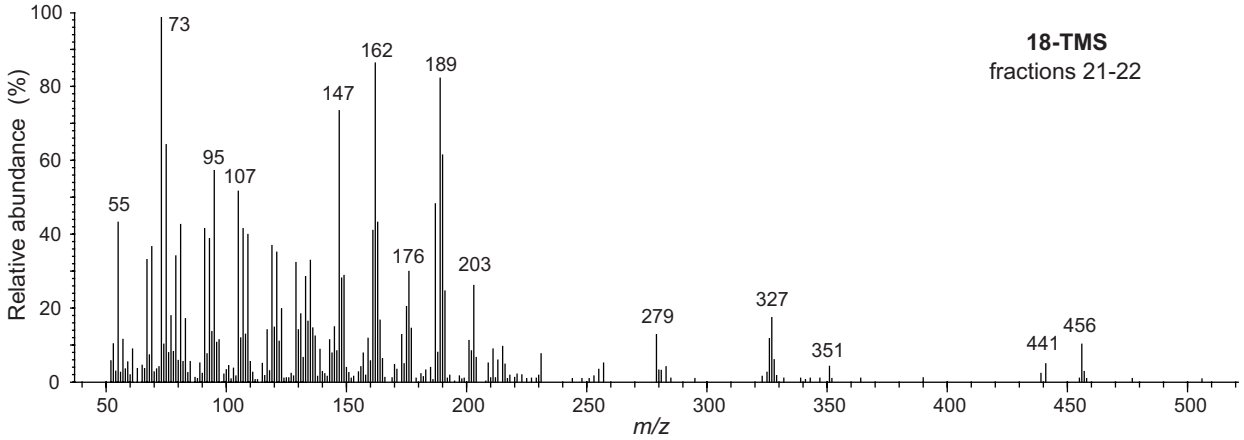

Figure S18. Mass spectrum of the TMS ether of trinorlupeol isomer **18**. Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da.

Analysis of nonpolar cuticular lipids: 3-keto triterpenoids

The first SPE fraction of the cuticular lipids contained no triterpenoids or sterols, only saturated long-chain lipids. The second fraction comprised mainly long-chain lipids, accompanied by ~0.1 mg of a 5:2:1 mixture of **5**, **6**, and **7**. No sterol or triterpenoid esters were detected (<10% of the amount of **5**) as judged by the absence of any NMR signal at δ_H 4.6 (sterols) or 4.51 (triterpenoids) corresponding to H3α.[16]

Characterization of **5**, **6**, and **7** (also detected in internal lipids): MS, Figure S19. GC-MS retention times for **5**, **6**, and **7** were 11.4, 14.1, and 15.7 min under GC conditions that gave retention times of 9.9, 12.0 and 13.2 min for TMS ethers of trinorlupeol, β-amyrin, and lupeol.[17] Partial ^{1}H NMR data ($CDCl_3$, 25 °C): **5**, δ (±0.001 ppm) 5.063 (br q, 2.2 Hz, H19), 1.774 (ddd, 12.4, 7.9, 1.8, H22β), 1.101 (d, 0.9 Hz, 8-Me), 1.003 (t, 0.6 Hz, 17-Me), 1.079 (s, 4α-Me), 1.037 (s, 4β-Me), 0.964 (d, 1.0 Hz, 10-Me), 0.786 (d, 0.7 Hz, 14-Me); **6**, δ (±0.003 ppm) 1.145 (d, 0.9

Hz, 14-Me), 1.097 (s, 4α-Me), 1.057 (s, 4β-Me), ~1.024 (d, 0.7 Hz, 10-Me), 0.842 (s, 17-Me); **7**, δ (±0.001 ppm) 4.573 (br s), 4.692 (br s); methyl signal positions were ambiguous.

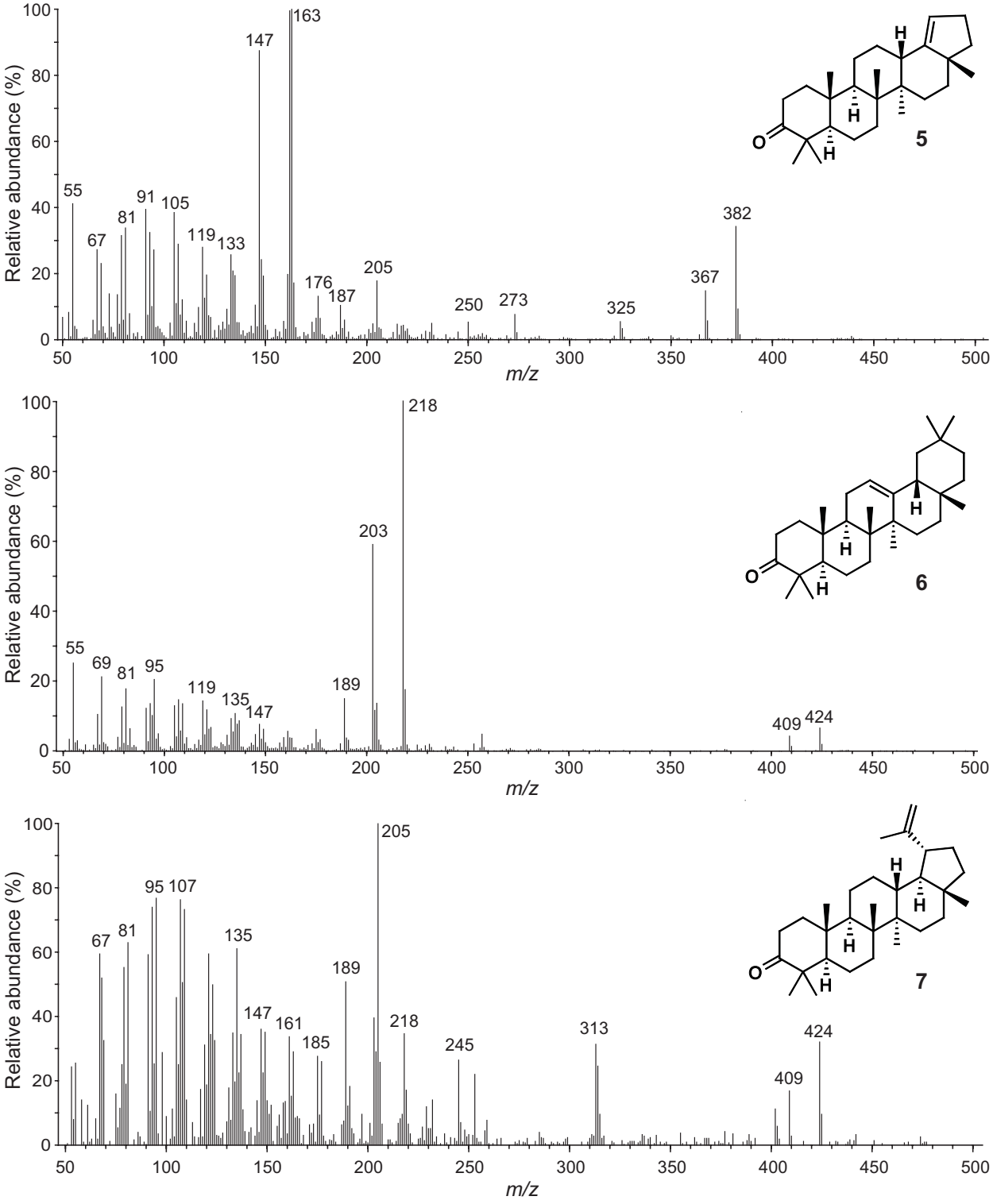

Figure S19. Mass spectra of 3-keto derivatives (**5**-**7**) of trinorlupeol, β-amyrin, and lupeol. Conditions: electron-impact ionization at 70 eV; 230 °C ion source; mass range of 50–650 Da.

Analysis of various cruciferous vegetables for trinorlupeol

β-Amyrin and α-amyrin have long been known in cabbage[18] and Brussels sprouts.[19] Recently, lupeol was also reported in cabbage, at 4-5 times the level of the amyrins.[20] Because of the association between trinorlupeol and lupeol biosynthesis in *A. thaliana*, we considered that trinorlupeol might be found in cultivars of cabbage (*Brassica oleracea*), such as Brussels sprouts and broccoli.

We analyzed several cruciferous vegetables for trinorlupeol and triterpene alcohols. Like *A. thaliana*, these vegetables are in the Brassicaceae family. However, as indicated in Table S7, the vegetables are not closely related to *A. thaliana*, which is in a different lineage.[21]

Table S7. Taxonomy of cruciferous vegetables studied herein

common name	**genus and species**	**classification within Brassicaceae**[a]
Brussels sprouts	*Brassica oleracea*	Brassicaea tribe (lineage II)
broccoli	*Brassica oleracea*	Brassicaea tribe (lineage II)
mustard greens	*Brassica juncea*	Brassicaea tribe (lineage II)
radish	*Raphanus sativus*	Brassicaea tribe (lineage II)
watercress	*Nasturtium officinale*	Arabideae tribe (lineage II)
horseradish	*Armoracia rusticana*	Arabideae tribe (lineage II)

[a] For comparison, *A. thaliana* is in the Camelineae tribe (lineage I).

Cruciferous vegetables were purchased from Whole Foods, a local Houston supermarket. Within 2 h of purchase, the vegetables were photographed (Figure S20), sampled, and soaked in hexane for about 1 h. The hexane extracts were evaporated to dryness and analyzed for triterpenoids by GC-MS using cholesteryl ethyl ether as an internal standard.

Broccoli was sampled as thin peelings from main stem (~0.5 mm thick, 10.9 g), intact branched stem (4.2 g), and floral buds (4.7 g). The main stem peelings contained modest amounts of α- and β-amyrin (0.2-0.7 μg/g fresh tissue) but no lupeol. The branched stem contained traces (0.1 μg/g fresh tissue) of α- and β-amyrin but no lupeol. In contrast, floral buds showed substantial amounts of α-amyrin, β-amyrin, lupeol, and other triterpene alcohols (1 – 10 μg/g fresh tissue). No trinorlupeol was observed.

Brussels sprouts were sampled as outer leaves (3.8 g) and stem (2.2 g and 4.1 g); tissue from the main stalk was not available. These samples contained roughly 0.5 μg/g each (range, 0.2 – 0.9 μg/g fresh tissue) of β-amyrin, α-amyrin, and lupeol.

No nonsterol triterpenoids were observed in mustard greens (sampled as thin peels of stem and as remaining inner tissue) or radish (sampled as thin slices of green stem, thin slices of red skin of the root, and inner root tissue). Thin slices of watercress stem and root tips contained only traces (<0.1 μg/g fresh tissue) of α- and β-amyrin and no lupeol. No nonsterol triterpenoids were detected in either the skin or inner tissue of horseradish root. Interestingly, the root skin contained elevated levels of cycloeucalenol and 24-methylenecycloartenol.

Figure S20. Pictures of cruciferous vegetables analyzed for trinorlupeol and triterpenes: broccoli (A), radish (B), horseradish (C), watercress (D), mustard greens (E), and Brussels sprouts (F).

Our results show that, except for trinorlupeol, the significant triterpenes of *A. thaliana* (β-amyrin, α-amyrin, and lupeol) are present in several vegetables. The low levels (<1 μg/g fresh tissue) in Brussels sprout and broccoli stem compared with levels in *A. thaliana* stem (4 - 20 μg/g fresh tissue for β-amyrin and lupeol) are largely attributable to the much greater size of the vegetable stems and thus a lower surface area relative to mass. Floral buds in broccoli and *A. thaliana* are more similar in size than their stems, and the levels of triterpenes were likewise more similar. The lack of triterpenoids in radish root and mustard leaves may reflect an organ-specific distribution of triterpenoids. For example, *A. thaliana*

stem contains far more triterpenoids than cauline and rosette leaves. In the vegetables, as well as in *A. thaliana*, sitosterol and campesterol were present at levels 10-100 times those of triterpenes.

In conclusion, no trace of trinorlupeol was observed in any sample of the cruciferous vegetables, whereas other triterpenes were found at plausible levels.

Analysis of birch bark for trinorlupeol

Peelings of birch bark were collected from white birch trees (presumably *Betula verrucosa*) in a forest in the Yaroslavl Oblast, near Yaroslavl, Russia. A 1 × 2 cm section of bark was immersed first in hexane for 3 min and then in MTBE for 30 min. The two extracts were evaporated to dryness, combined, and analyzed by ^{1}H NMR and GC-MS. Spectral analyses indicated a 1:5 mixture of lupeol and betulin (28-hydroxylupeol), with at most minor amounts of other neutral triterpenoids. Trinorlupeol was not observed at a detection limit of 0.2% of the major triterpenoid betulin. Relevant portions of the NMR spectrum are shown in Figure S21.

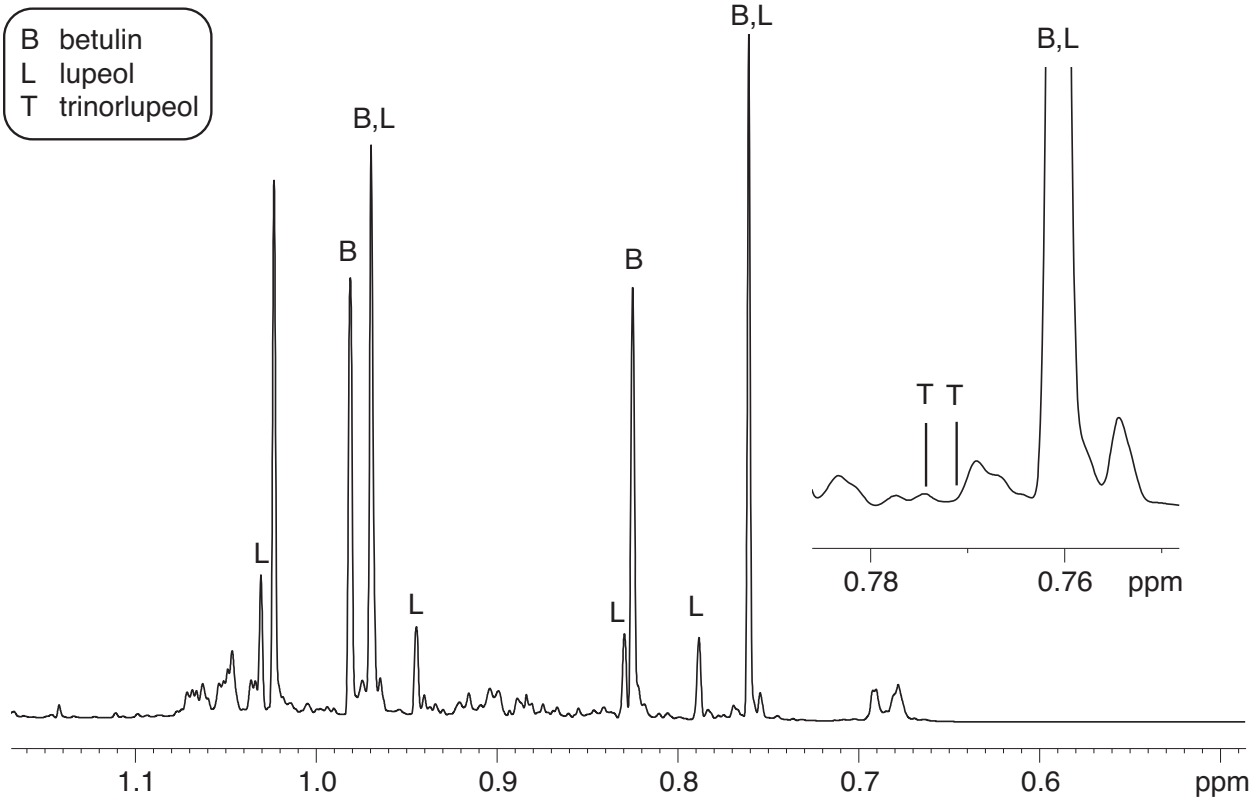

Figure S21. ^{1}H NMR spectrum of birch bark extracts. Conditions: 800 MHz, 25 °C sample temperature, ~10 mM triterpenoids in $CDCl_3$.

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(14) Samples of β-amyrin, lupeol, and cholesteryl ethyl ether from Sigma-Aldrich were used to make the calibration curves. NMR spectra (800 MHz) showed ~99% purity for the lupeol

sample (containing 0.5% β-amyrin and minor non-triterpene impurities) and ~98% purity for the β-amyrin sample (containing 1.5% α-amyrin, 0.1% lupeol, and minor non-triterpene impurities). A combined trinorlupeol sample, which had been used for 2D NMR analysis, evaporated to dryness, and then used for the calibration curves, contained about 5% grease and about 5% other impurities, including decomposition products from exposure to the NMR solvent ($CDCl_3$). (The ^1H NMR spectrum in Figure S6 was obtained from a more highly purified sample of trinorlupeol.) An 800 MHz ^1H NMR spectrum of cholesteryl ethyl ether showed 92% purity: 1% cholesteryl chloride, 5% cholesteryl methyl ether, and 1-2% unidentified sterols. The calibration curves were corrected for the triterpenoid impurities, which appeared not to coelute on GC with the respective major component. Because a single batch of the internal standard was used throughout this work, the impurities in the cholesteryl ethyl ether sample did not affect quantification of the triterpenoids. In separate experiments, mixtures of trinorlupeol, β-amyrin, lupeol, and cholesteryl ethyl ether were quantified by GC-FID, GC-MS, and ^1H NMR to determine the relative responses of these compounds. The results indicated the calibration curves in Figure S11 were fundamentally sound.

(15) The least squares line was forced to pass through the origin. Weighted regression was not performed because the close fit of points to the line in the unweighted calculation suggested that weighting would offer negligible improvement in estimating the slope.

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