Electronic Supplementary Material for

Biosynthesis of Acorane Sesquiterpenes by *Trichoderma*

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Table 1 Volatiles identified in the headspace extracts of three strains of *Trichoderma*.

Compound[a]	*I*[b]	*I* (Lit.)[c]	*T. harzianum* 714[d]		*T. longibrachiatum* 594		*T. viride* 54	
3-Methylbutan-1-ol				x			x	xxx
2-Methylbutan-1-ol					x		x	xx
Isobutyl acetate					x			
2,3-Butanediol					x			x
3-Methylbutyl acetate	882							x
2-Methylbutyl acetate	884						x	x
Oct-1-en-3-ol	980		x	x	x			x
Octan-3-one	987		x	x	x		x	x
Octan-3-ol	996							x
4-Methylhex-5-en-4-olide	1054				x			
Linalool oxide	1087				x			
Linalool	1101		x	x	x			
2-Phenylethanol	1115		x	x	x		xx	xxx
Camphor	1143							x
α-Terpineol	1192		x					x
3,4-Dimethoxystyrene	1370	1379[3]	x					
2-Acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one	1383			x	x			x
(–)-α-Funebrene (**11**)	1383	1385[1]	x	x	x	x	x	x
(–)-β-Funebrene (**8**)	1418	1418[1]	xx	xx	x	x	xx	x
Acora-2,4-diene (**3**)	1427		xx	xx	xx	xx	xx	xx
(–)-β-Cedrene (**7**)	1429	1424[1]	xx	xx	x	x	xxx	x
β-Duprezianene (**9**)	1431	1424[1]	xx	x			xx	
Acora-3,5-diene (**4**)	1433	1421[1]	xx	xx	xx	x	x	xx
Sesquisabinene B (**12**)	1447	1446[1]	x	x	x	x	x	x
epi-Isozizaene (**13**)	1451		x	x	x	x	x	x
Myltayl-8(12)-ene (**14**)	1453	1445[2]	x	x		x	x	
Cadina-4,11-diene (**15**)	1455	1446[1]	x	x	x	x	x	x
(*E*)-β-Farnesene (**16**)	1457	1446[1]		x	x			x
Muurola-4,11-diene (**17**)[e]	1461	1458[1]	x	x		x	x	
Amorpha-4,11-diene (**18**)	1463	1472[1]	x	x	x	x	x	x
Muurola-4(15),5-diene (**19**)	1468	1467[2]	x	x	x	x	x	x
(+)-β-Microbiotene (**20**)	1471	1473[1]	x	x	x	x	x	x
γ-Muurolene (**21**)	1475	1474[1]	x	x	x	x	x	x
α-Neocallitropsene (**5**)	1477	1475[1]	xx	xx	x	x	xx	x
Unknown (MS similar to **20**)	1480		x	x	x	x	x	x
α-Curcumene (**22**)	1485	1473[1]	x	x	x	x	x	x
Bicyclosesquiphellandrene (**23**)	1486	1487[1]					x	
Zingiberene (**24**)	1496	1489[1]			x			
β-Alaskene (**25**)	1499	1495[1]	x	x			x	
10-*epi*-Zonarene (**26**)	1502	1494[1]				x		
α-Cuprenene (**27**)	1505	1497[1]	x	x			x	
(*Z*)-α-Bisabolene (**28**)	1506	1494[1]	x		x	x	x	x
α-Chamigrene (**29**)	1508	1503[1]	x	x			x	
(*S*)-Cuparene (**30**)	1511	1498[1]	x	x	x	x	x	x
(*S*)-β-Bisabolene (**31**)	1513	1503[1]	x	x	x	x	x	x

α-Alaskene (**6**)	1517	1512[1]	xx	xx	x	x	xx	x
cis-Calamenene (**32**)	1528	1517[1]	x	x	x	x	x	x
β-Sesquiphellandrene (**33**)	1528	1516[1]	x	x	x	x	x	x
Zonarene (**34**)	1531	1521[1]	x	x	x	x	x	
(*S*)-γ-Cuprenene (**35**)	1536	1523[1]	x			x	x	
trans-Calamenene (**36**)	1539	1530[1]	x	x	x	x	x	x
δ-Cuprenene (**37**)	1549	1546[1]	x	x	x	x	x	x
(*E*)-Nerolidol (**10**)	1567	1561[2]	x	x	xxx	xxx	x	x
Tricho-acorenol (**1**)	1673		xxx	xxx	xxx	xxx	xxx	xxx
Acorenone (**2**)	1720		xxx	xx	xx	xx	x	x

[a] Other unidentified compounds, artifacts, and medium compounds are not mentioned. [b] Determined from a homologous series of alkanes (C_8 – C_{36}). [c] Literature data obtained on the same (HP-5) or similar GC column (DB-5, CpSil-5). [d] Relative amounts are indicated by x: < 2%, xx: 2 – 8%, xxx: >8% of total area in the total ion chromatogram. [e] May also be 10-*epi*-muurola-4,11-diene (**38**) or a mixture of **17** and **38** that have identical mass spectra and retention indices.[4]

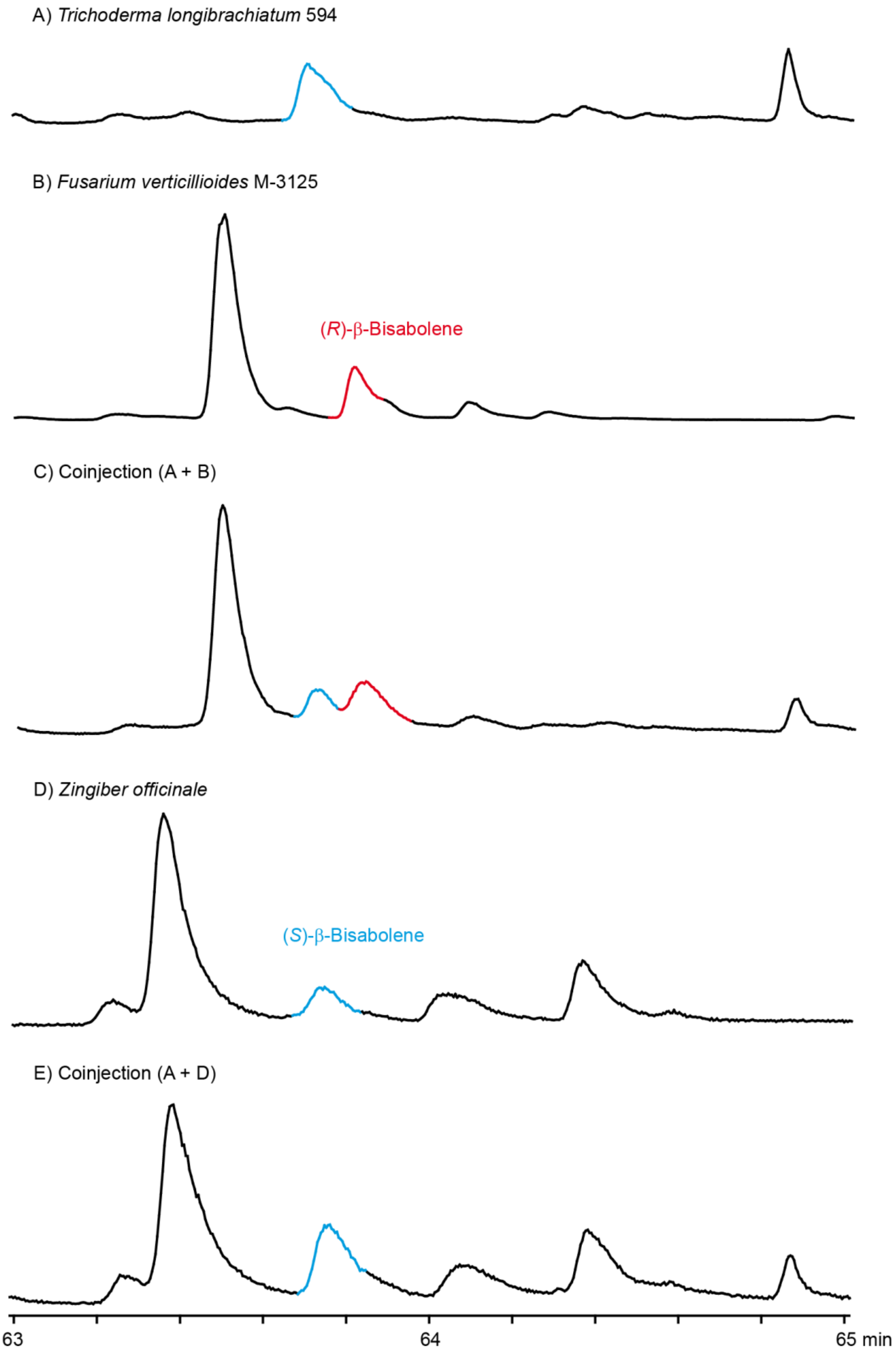


Figure 1 Total ion chromatograms of the headspace extracts of A) *Trichoderma longibrachiatum* 594, B) *Fusarium verticillioides* M-3125 containing (*R*)-β-bisabolene (N. L. Brock, B. Tudzynski, J. S. Dickschat, unpublished results), C) a mixture of the headspace 5 extracts from *T. longibrachiatum* 594 and *F. verticillioides* M-3125, D) the essential oil from *Zingiber officinale* containing (*S*)-β-bisabolene,[5] and E) a mixture of the headspace extract from *T. longibrachiatum* 594 and the essential oil from *Zingiber officinale* on a chiral hydrodex GC column. The coinjection experiment C) shows two separated peaks for β-bisabolene, whereas experiment E) gives only one peak. Conclusively, *Trichoderma longibrachiatum* 594 produces the same enantiomer as *Zingiber officinale*, i. e. (*S*)-β-bisabolene.

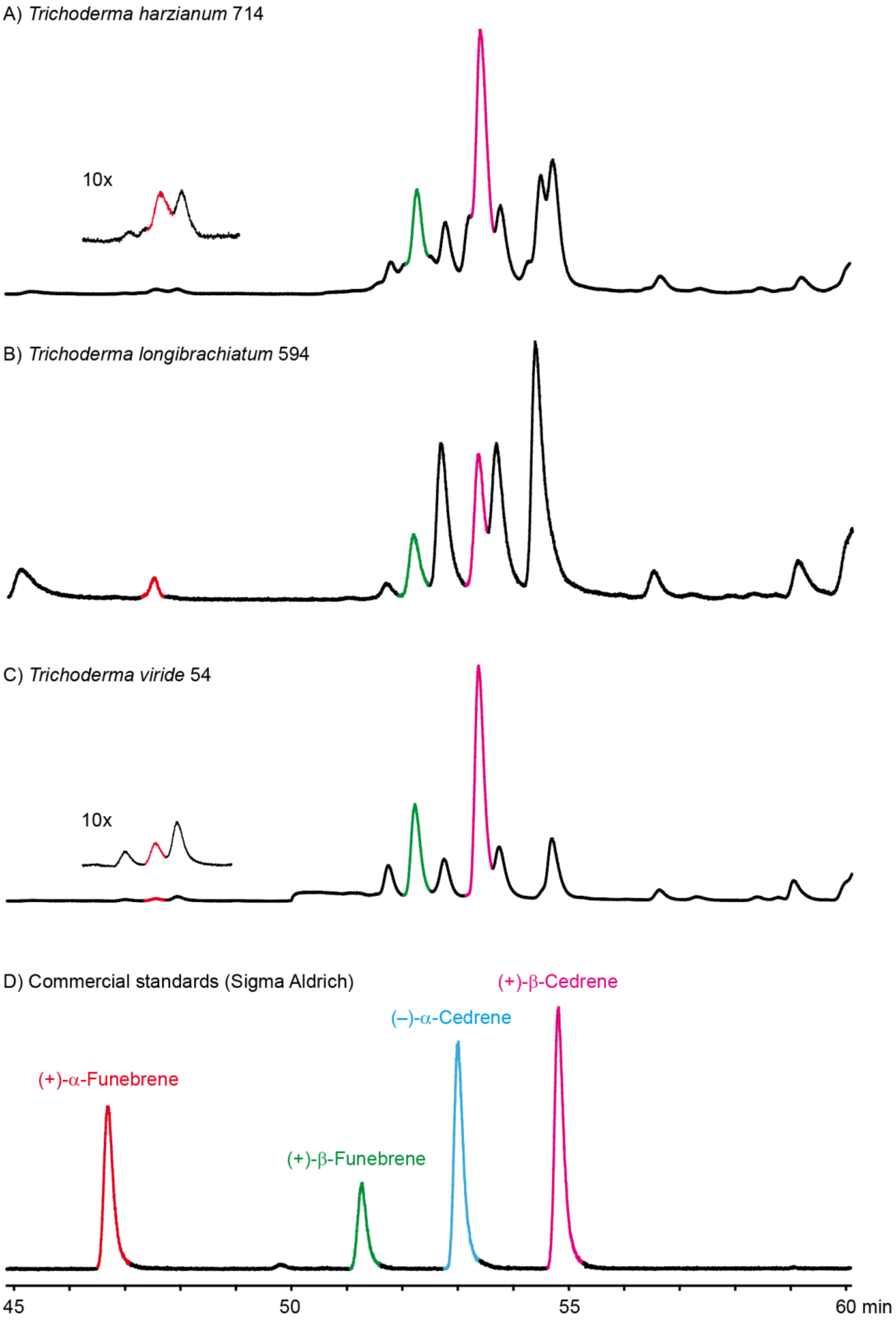


Figure 2 Total ion chromatograms of the headspace extracts of A) *Trichoderma harzianum* 714, B) *T. longibrachiatum* 594, C) *T. viride* 54, and D) a mixture of commercial standards of (+)-α-funebrene (red), (+)-β-funebrene (green), (–)-α-cedrene (blue), and (+)-β-cedrene (purple) on a chiral hydrodex GC column. The compounds α-funebrene, β-funebrene, and β-cedrene from all *Trichoderma* strains elute with retention times that differ from the retention times of the respective standards, whereas α-cedrene is not found in the *Trichoderma* extracts. In conclusion, these compounds are produced as the antipodes (–)-α-funebrene, (–)-β-funebrene, and (–)-β-cedrene by *Trichoderma* that only rarely occur in Nature, but have been reported from mosses.[6]

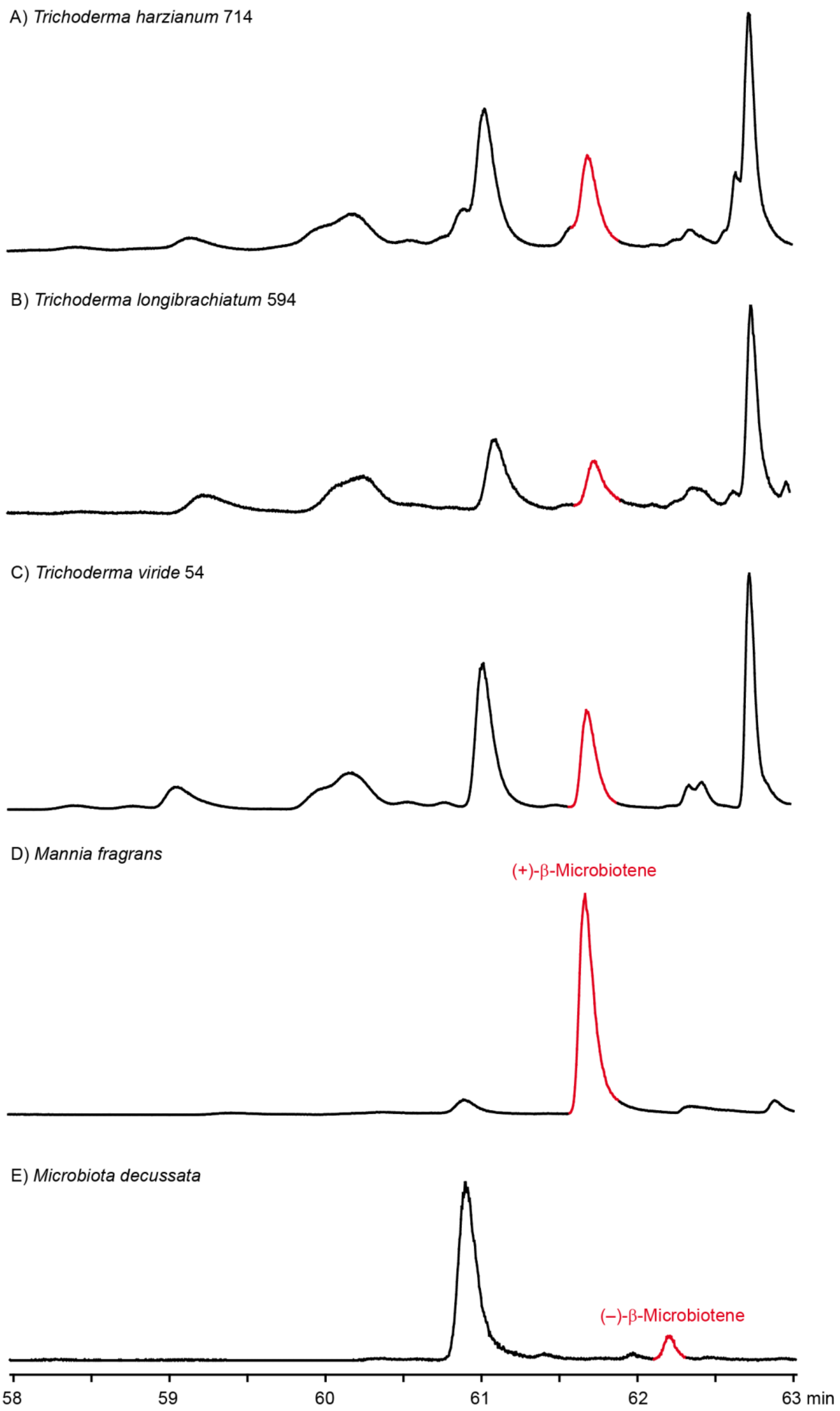


Figure 3 Total ion chromatograms of the headspace extracts of A) *Trichoderma harzianum* 714, B) *Trichoderma longibrachiatum* 594, C) *Trichoderma viride* 54, D) the essential oil of the liverwort *Mannia fragrans* containing (+)-β-microbiotene, and E) the essential oil of *Microbiota decussata* containing (–)-β-microbiotene.[7] The β-microbiotene from *Trichoderma* elutes with the same retention time as (+)-β-microbiotene from *M. fragrans*.

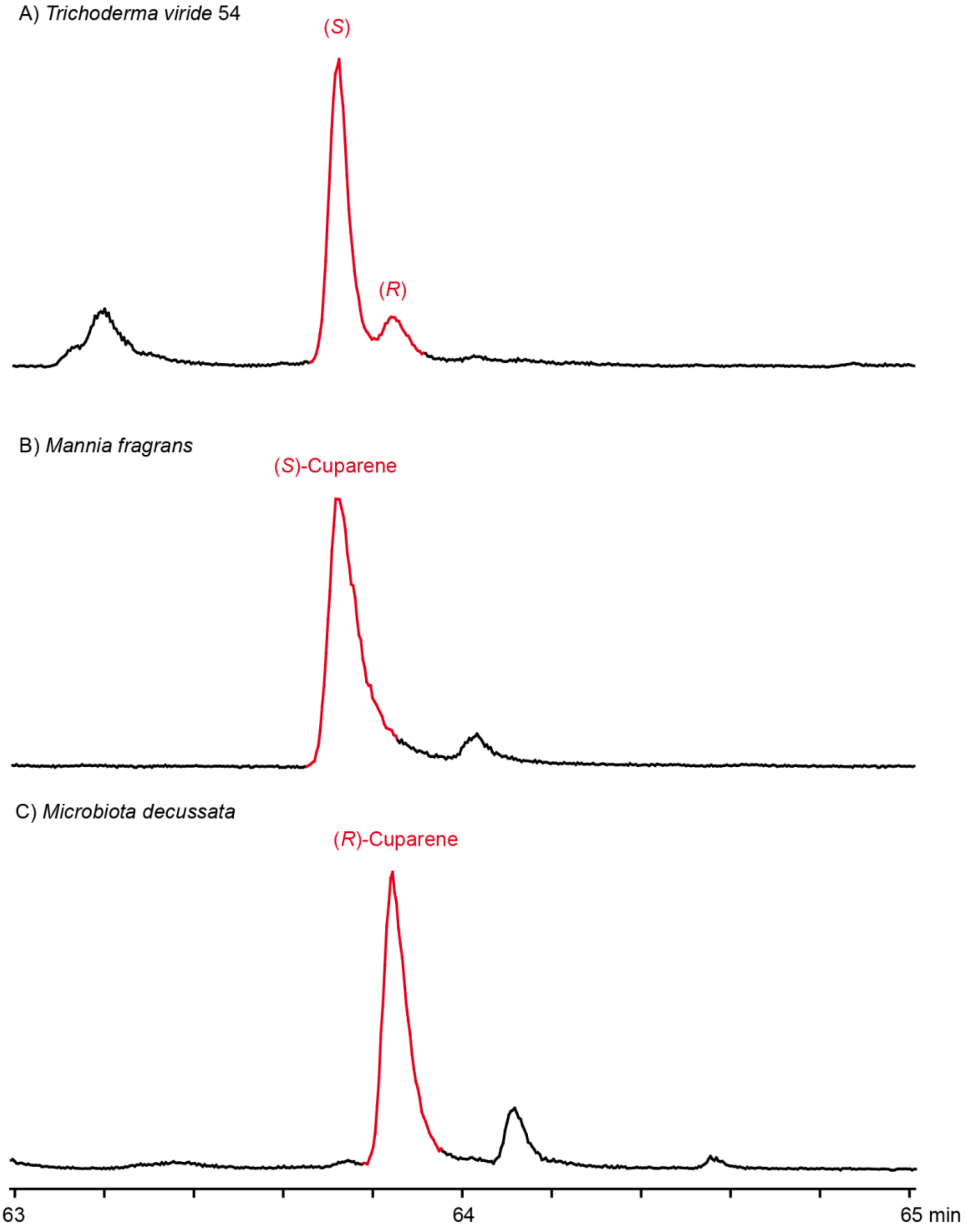


Figure 4 Total ion chromatograms of the headspace extracts of A) *Trichoderma viride* 54, B) the essential oil of the liverwort *Mannia fragrans* containing (*S*)-cuparene, and C) the essential oil of *Microbiota decussata* containing (*R*)-cuparene. The absolute configurations of cuparene from *M. fragrans* and *M. decussata*, respectively, were deduced from the known absolute configurations of structurally related β-microbiotene.[7] The cuparene from *T. viride* is a mixture of enantiomers mainly composed of (*S*)-cuparene (67% *ee* determined by GC).

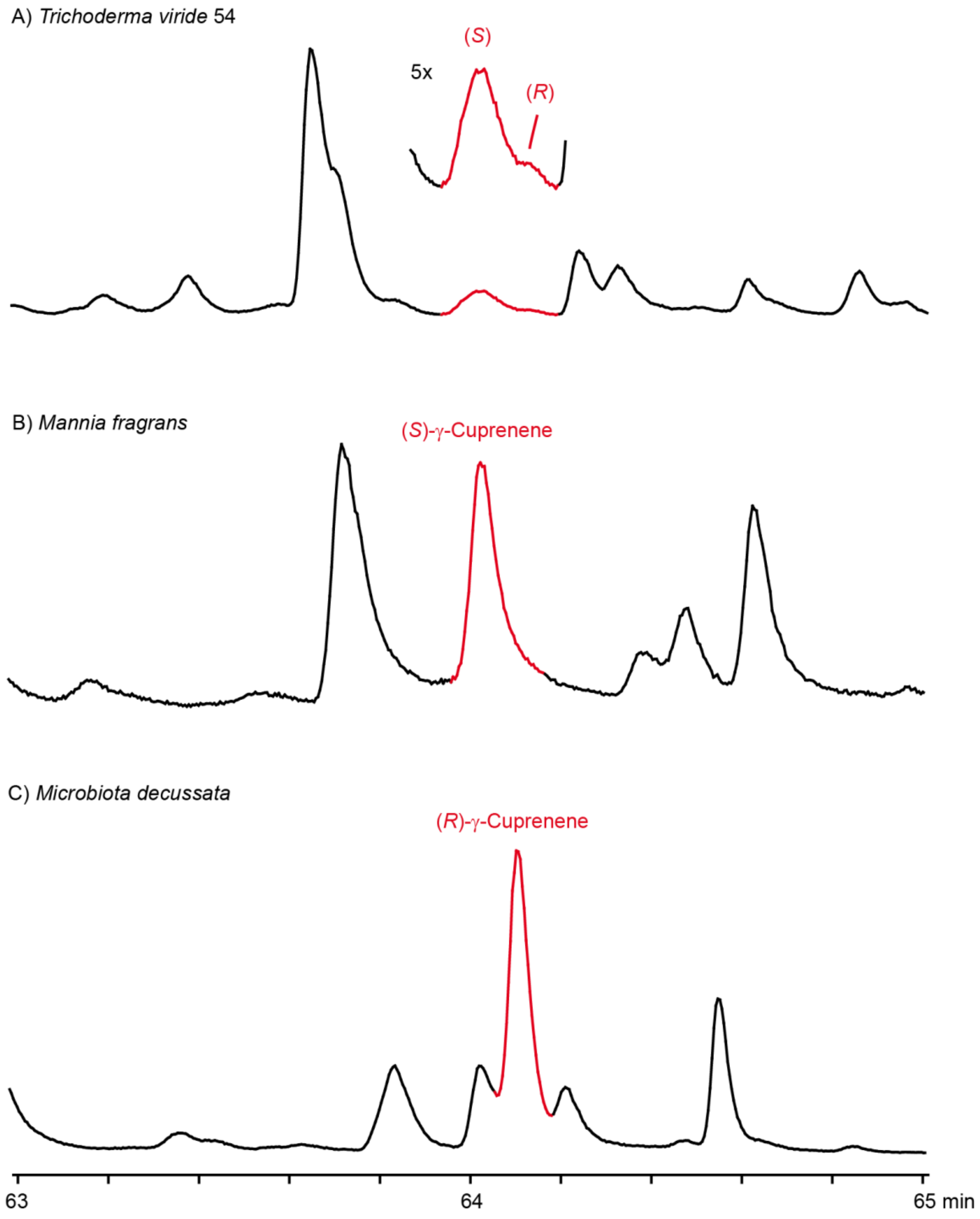


Figure 5 Total ion chromatograms of the headspace extracts of A) *Trichoderma viride* 54, B) the essential oil of the liverwort *Mannia fragrans* containing (*S*)-γ-cuprenene, and C) the essential oil of *Microbiota decussata* containing (*R*)-γ-cuprenene. The absolute configurations of cuprenene from *M. fragrans* and *M. decussata*, respectively, were deduced from the known absolute configurations of the structurally related β-microbiotene.[7] The cuprenene from *T. viride* is a mixture of enantiomers mainly composed of (*S*)-γ-cuprenene (determination of the *ee* was not possible due to the small amounts of material in the *Trichoderma* extract).

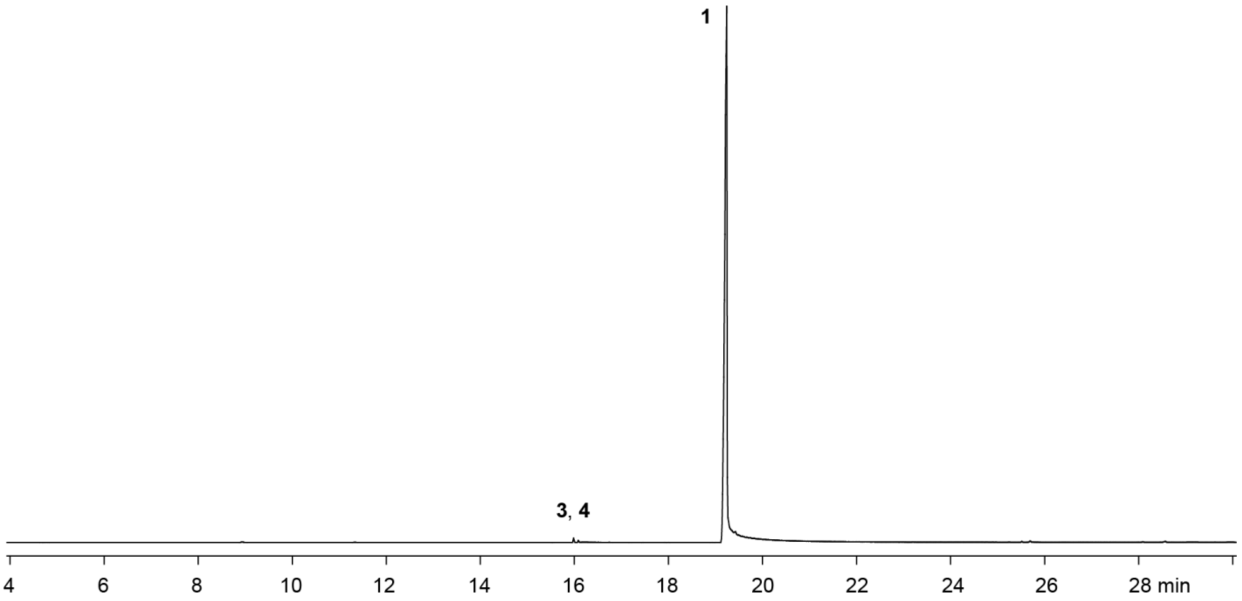
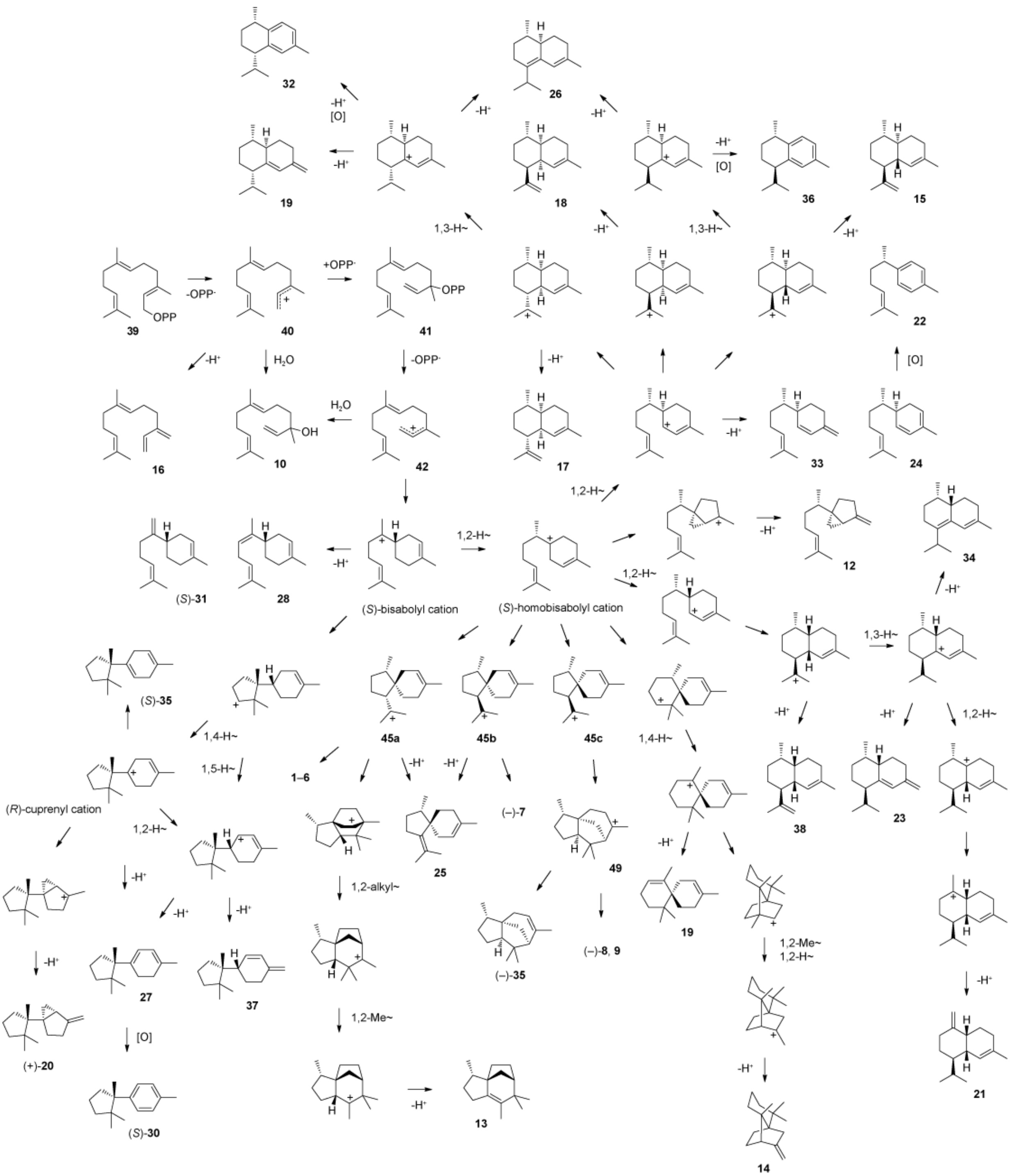


Figure 6 Total ion chromatogram of a GC-MS analysis of analytically pure (^{1}H NMR spectroscopy) tricho-acorenol (**1**). Small amounts (<0.2% of total integral in GC) of **3** and **4** are formed from **1** likely by a thermal reaction in the injection port of the GC.



Scheme 1 Biosynthetic pathway to all sesquiterpenes identified in the headspace extracts of three strains of *Trichoderma* (cf. main text for the biosynthesis of the main compounds **1** – **9**). The absolute configurations of compounds that have not been analysed by chiral GC were deduced from sesquiterpenes with identified absolute configuration. The biosynthetic scheme demonstrates that all cyclic sesquiterpenes are generated via the (*S*)-bisabolyl cation suggesting that a single terpene cyclase is responsible for the formation of all sesquiterpenes identified in the headspace extracts of *Trichoderma*. The compounds **30** and **35** were not found as pure enantiomers, but as enantiomeric mixtures (67% *ee*). This is explainable by a conversion of the (*S*)-bisabolyl cation not to the (*R*)-cuprenyl cation as shown in the scheme, but to the (*S*)-cuprenyl cation that serves as precursor for the minor enantiomers of **30** and **35**. This process may also result in the formation of sesquiterpenes that are epimers of **20** or **37**, but our compound libraries do not include the mass spectra and retention indices of these compounds. In fact, one additional compound with a mass spectrum very similar to the mass spectrum of β-microbiotene and a retention index of *I*=1480 (on a HP-5 column) was found in the *Trichoderma* extracts (the mass spectrum is shown in Figure 7 of SI). An alternative mechanism for the formation of the cuprenyl from the bisabolyl cation is discussed by Hong and Tantillo.[8]

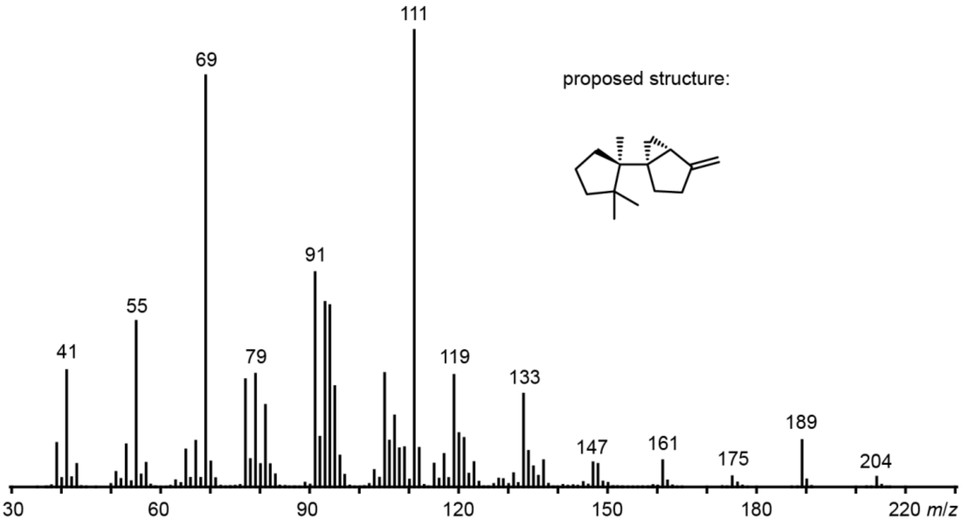


Figure 7 Mass spectrum of an unknown compound (*I*=1480) from *Trichoderma*. Due to the high similarity of the mass spectrum to the mass spectrum of β-microbiotene and biosynthetic considerations the respective compound may be the epimer of β-microbiotene.

References

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