

SUPPLEMENTARY MATERIAL

Phytochemistry meets geochemistry—Blumenol C sulfate: A new megastigmane sulfate from *Palicourea luxurians* (Rubiaceae: Palicoureeae)

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Blumenol C sulfate (1)

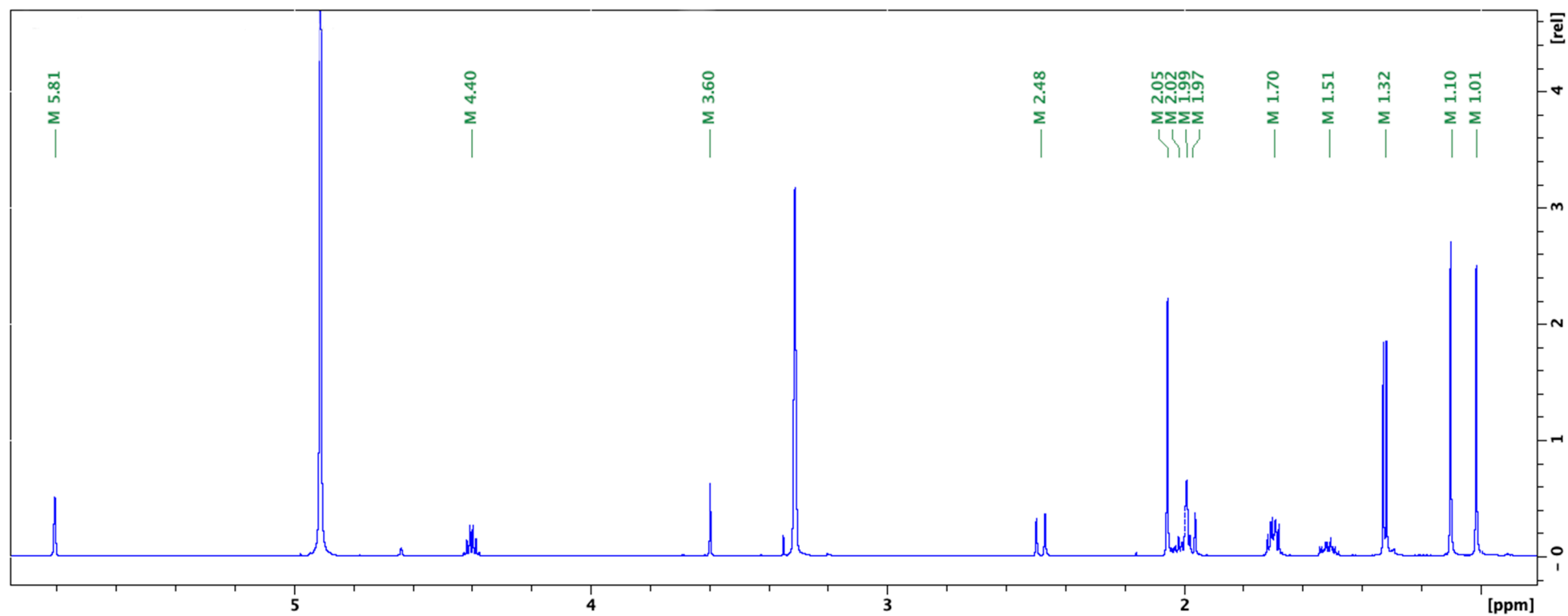


Figure S1. ¹H NMR of **1** in CD₃OD.

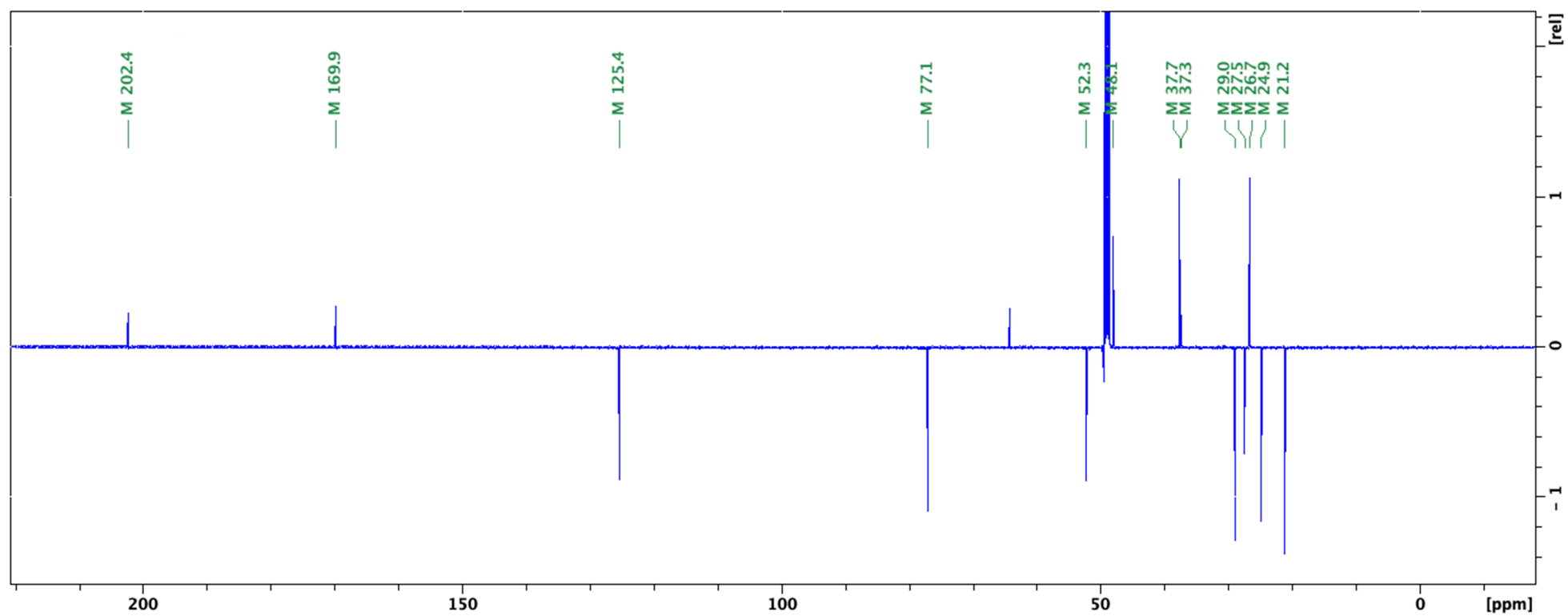


Figure S2. ¹³C NMR [APT] of **1** in CD₃OD.

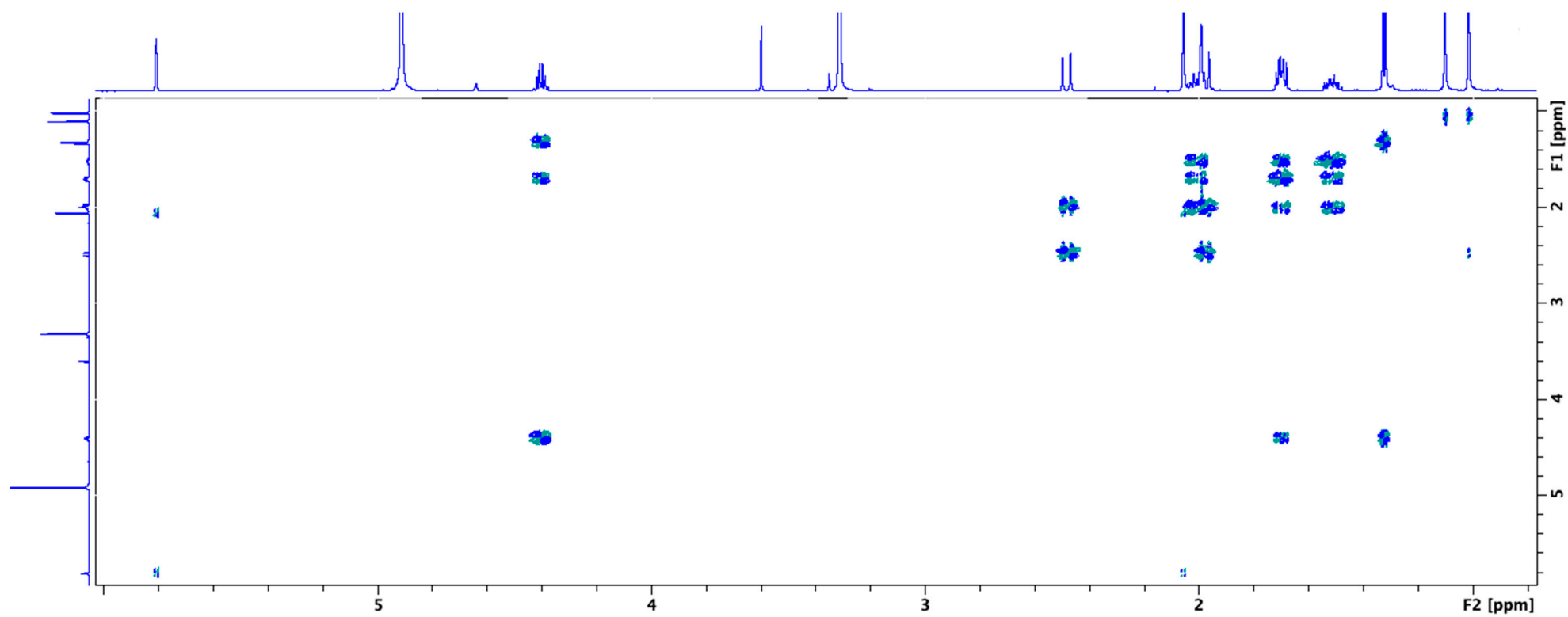


Figure S3. COSY of **1** in CD₃OD.

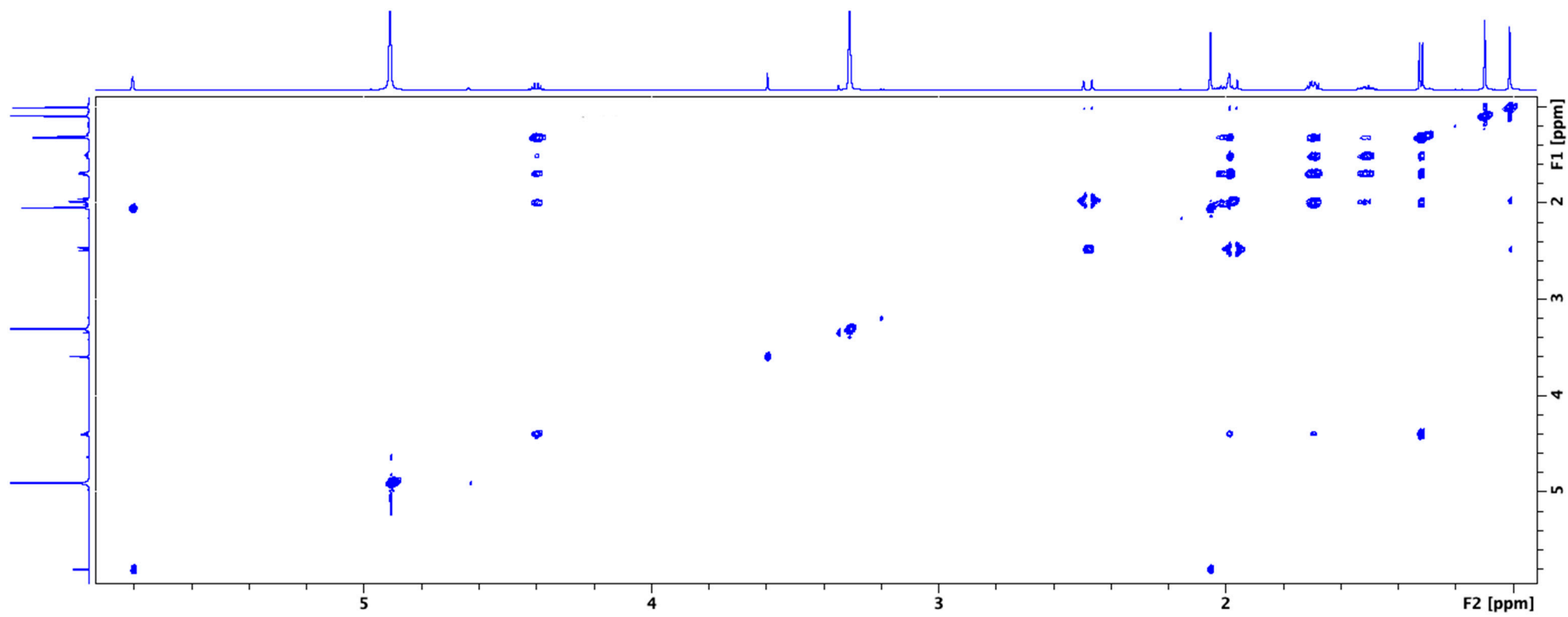


Figure S4. TOCSY of **1** in CD₃OD.

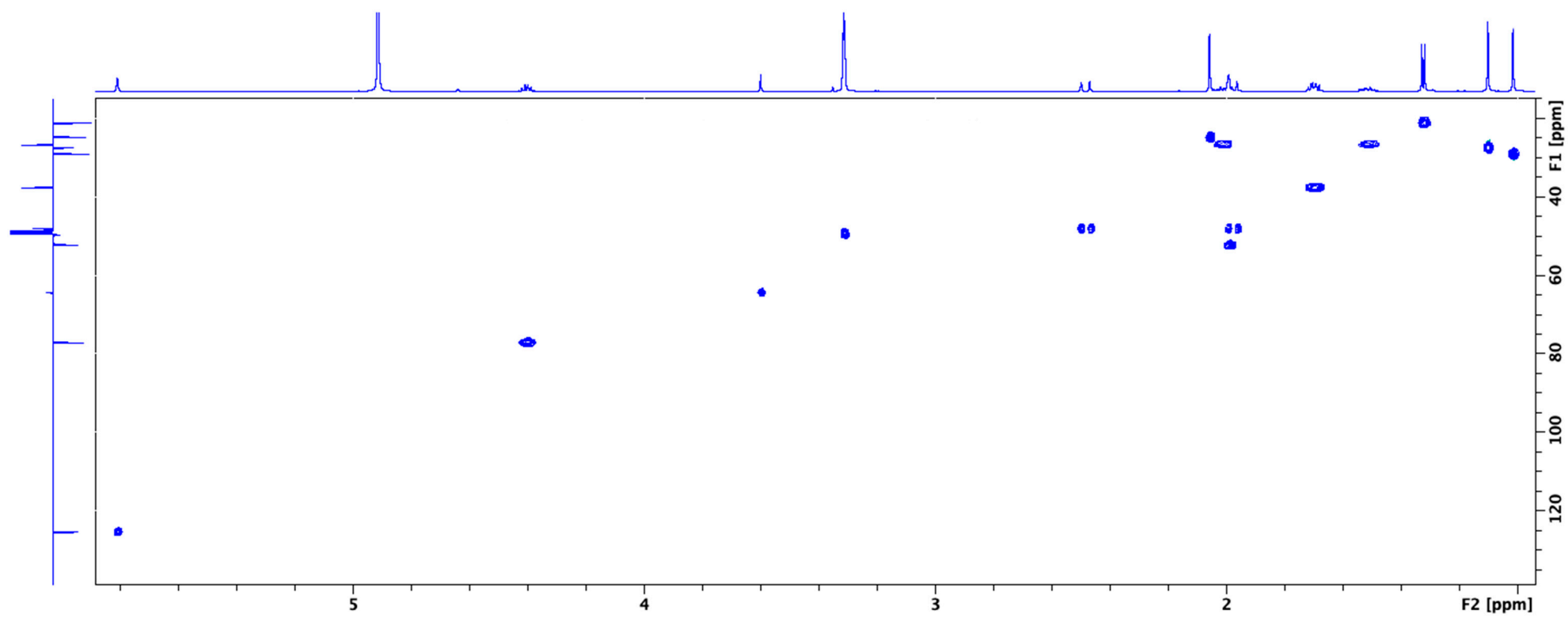


Figure S5. HSQC of **1** in CD₃OD.

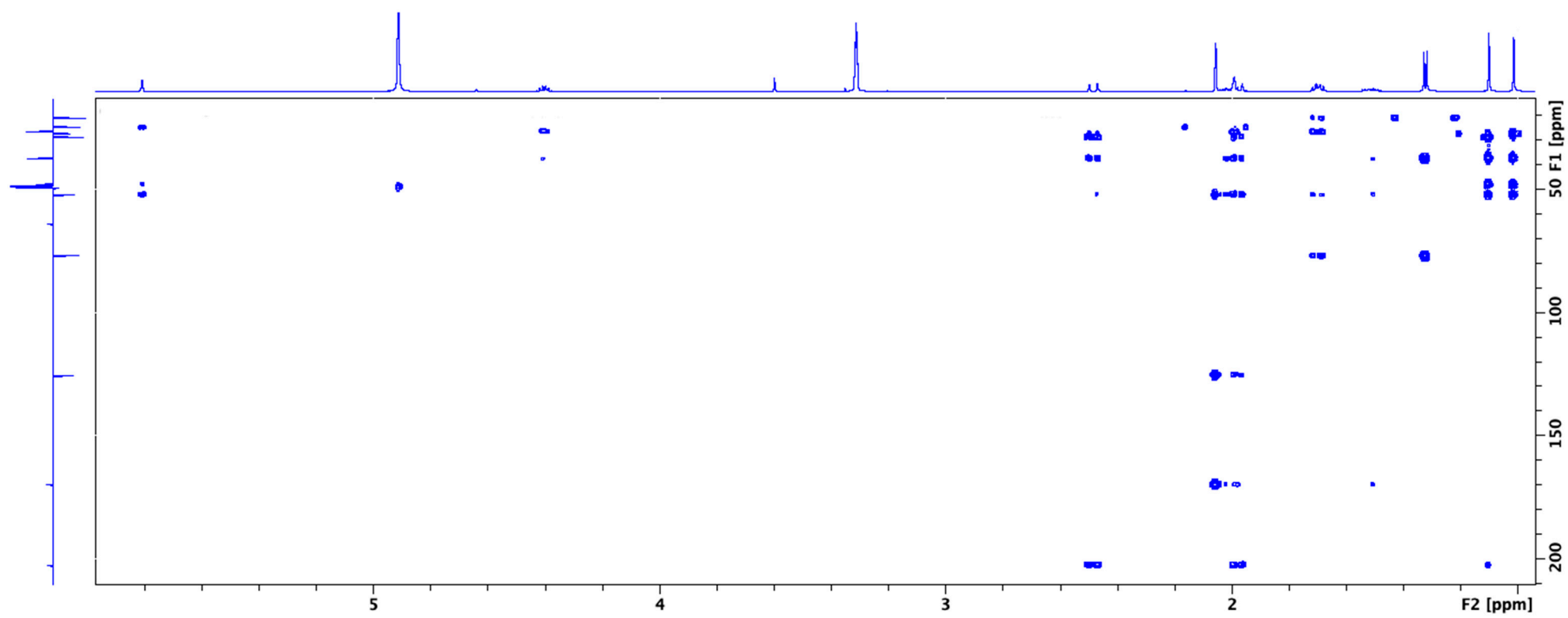


Figure S6. HMBC of **1** in CD_3OD .

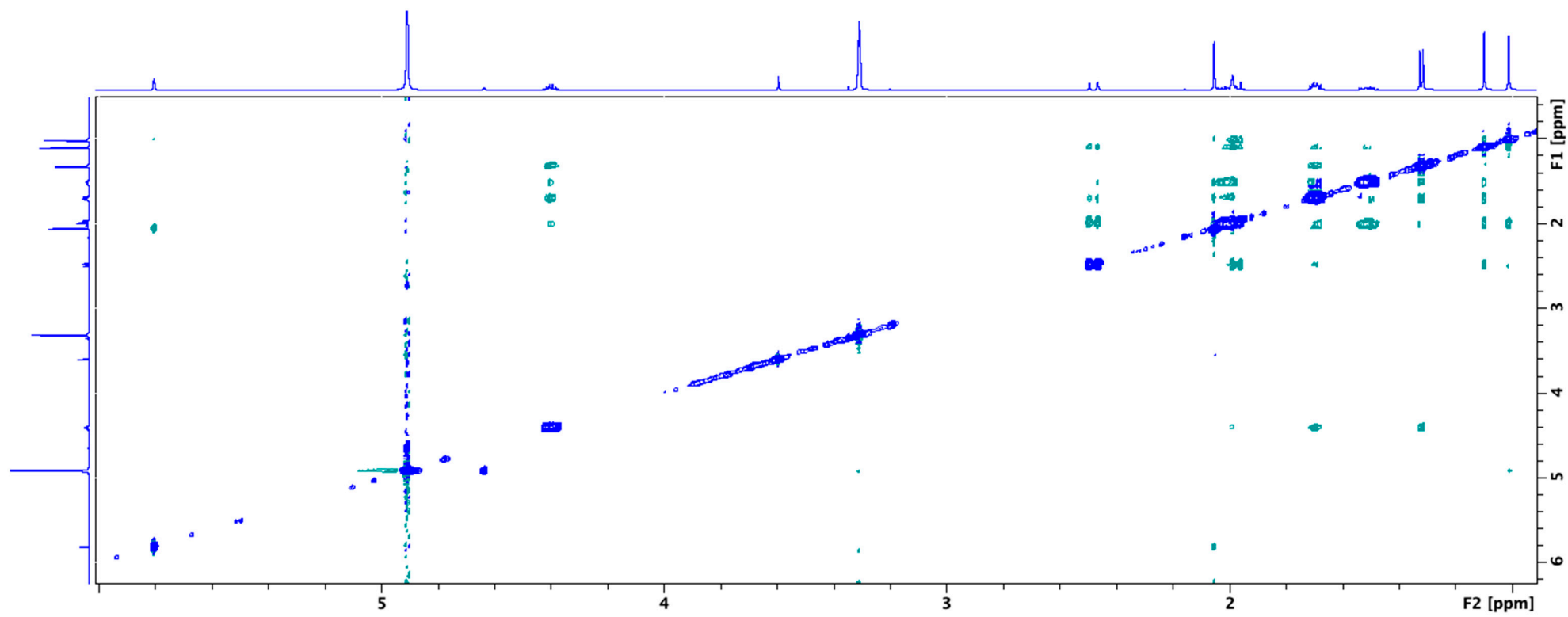


Figure S7. NOESY of **1** in CD₃OD.

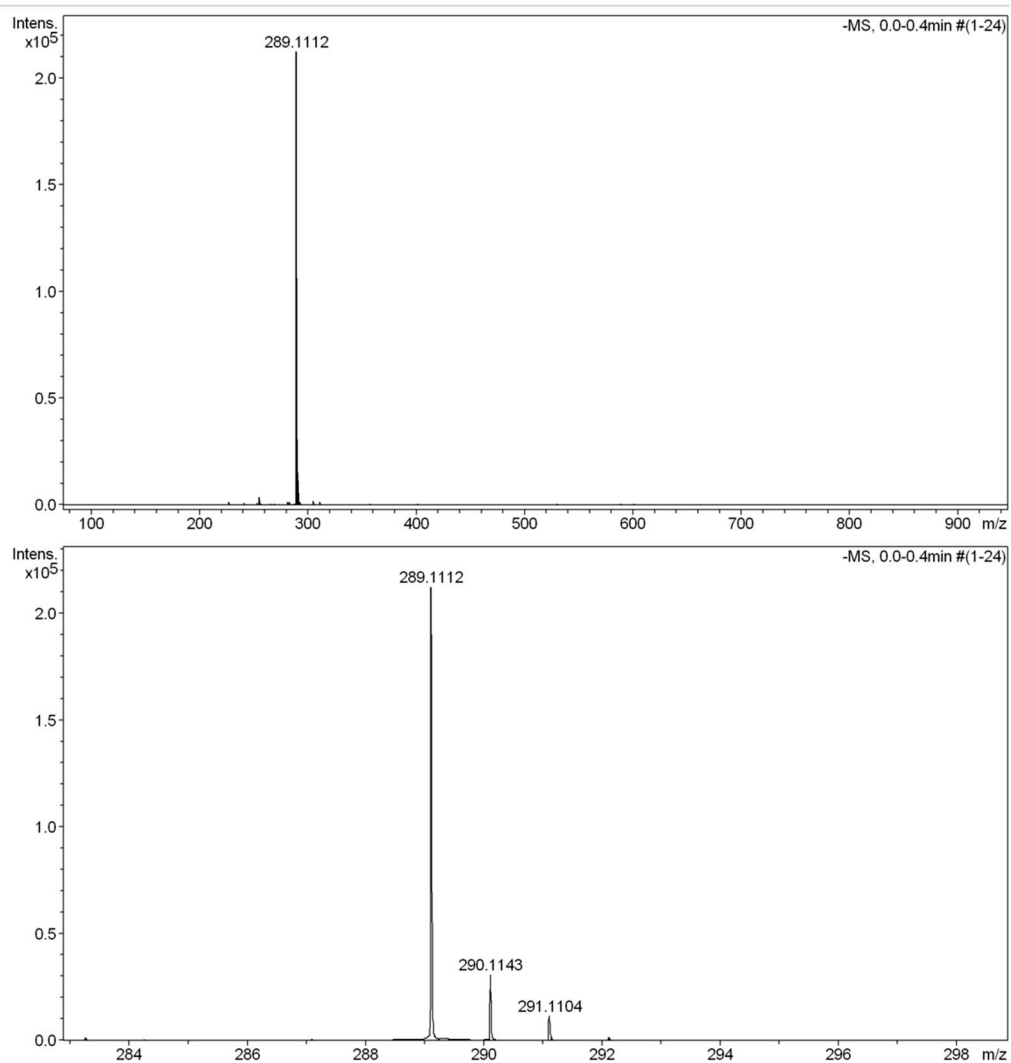


Figure S8. HR-ESI-TOF-MS (negative mode) of **1**.

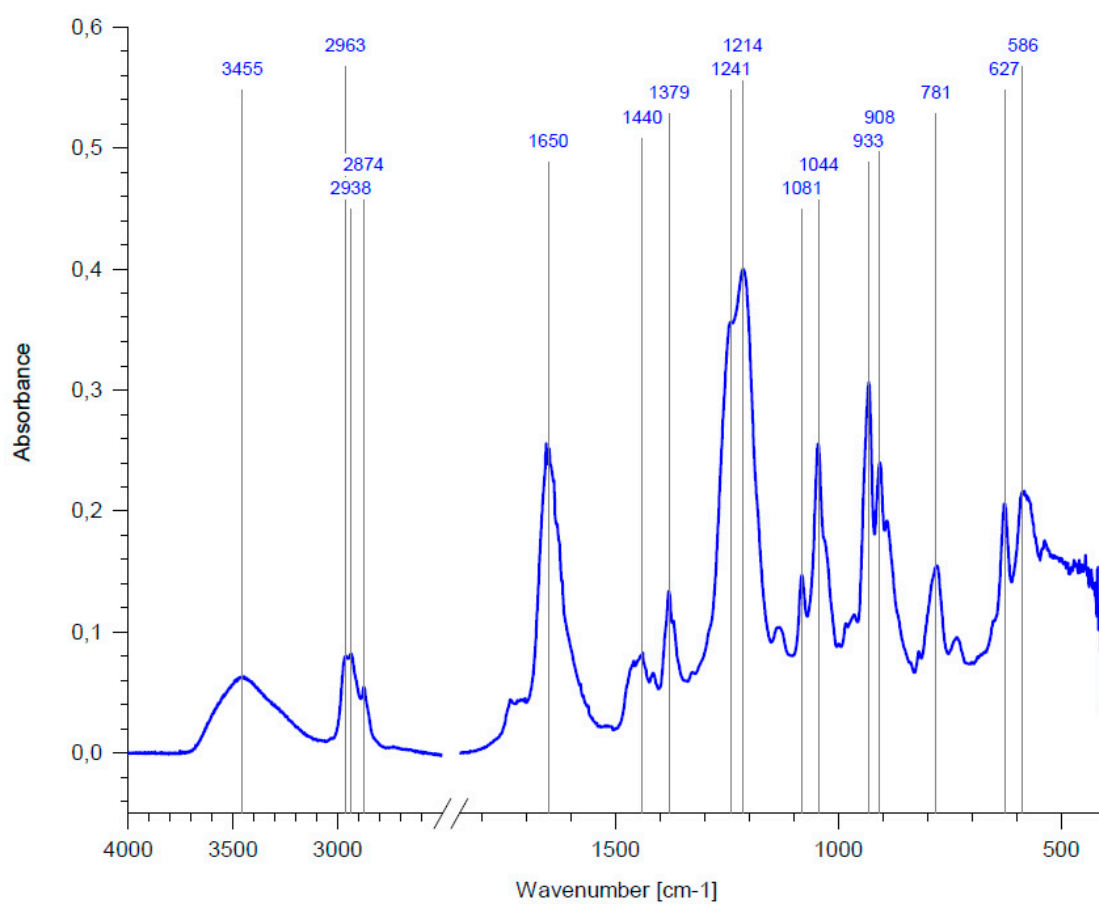


Figure S9. FT IR spectrum of **1**.

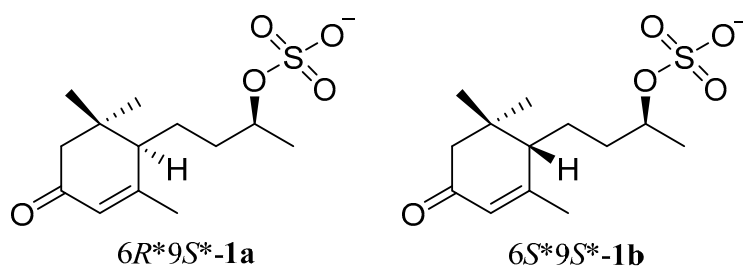


Figure S10. Two representative diastereomeric forms **6*R**9*S**-1a** and **6*S**9*S**-1b** used for conformation search based on molecular mechanics with MMFF force fields.

Table S1. DP4+ analysis results of **6*R**9*S**-1a** (Isomer 1) and **6*S**9*S**-1b** (Isomer 2).

Input	Isomer 1 (%)	Isomer 2 (%)
sDP4+ (H data)	44.69	55.31
sDP4+ (C data)	2.36	97.64
sDP4+ (all data)	1.92	98.08
uDP4+ (H data)	50.57	49.43
uDP4+ (C data)	50.37	49.63
uDP4+ (all data)	50.94	49.06
DP4+ (H data)	45.25	54.75
DP4+ (C data)	2.39	97.61
DP4+ (all data)	1.99	98.01

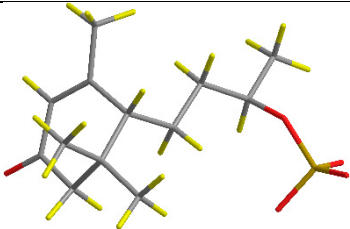
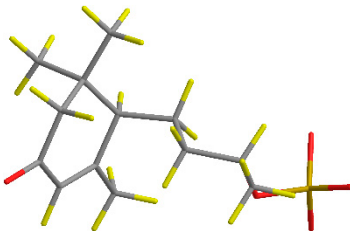
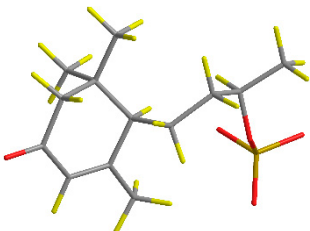
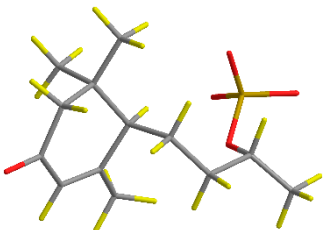
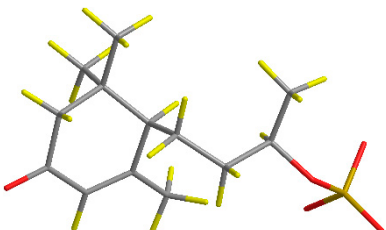
*Isomers 1 and 2 correlate to Figure S10

Table S2. Correlations between calculated (DP4+) and experimental ^1H and ^{13}C NMR chemical shifts of 6*R**9*S**-**1a** (Isomer 1) and 6*S**9*S**-**1b** (Isomer 2).

No.	^{13}C NMR chemical shifts (ppm)			^1H NMR chemical shifts (ppm)		
	Exp.	Isomer 1	Isomer 2	Exp.	Isomer 1	Isomer 2
1	26.7	29.1	28.0	1.99	2.52	2.58
2	29	30.0	28.8	2.48	2.76	2.94
3	37.7	35.6	35.3	1.97	2.26	2.45
4	77.1	85.6	88.2	5.81	6.42	6.20
5	21.2	23.1	21.8	1.10	1.40	1.38
6	48.1	49.0	48.6	1.01	1.28	1.28
7	202.4	198.1	197.3	2.05	2.41	2.49
8	125.4	119.3	123.0	1.51	2.16	1.94
9	169.9	174.6	171.5	2.02	2.11	2.14
10	52.3	50.8	52.0	1.69	1.96	1.92
11	37.3	42.8	40.7	4.40	4.72	4.67
12	27.5	29.7	27.7	1.32	1.68	1.68
13	24.9	27.8	28.3	-	-	-

*Isomers 1 and 2 correlate to Figure S10

Table S3. M06-2X/Def2SVP optimized lowest energy 3D conformers and energy analysis for compound **1**.

No.	3D conformers	G (Hartree)	ΔG (Kcal/mol)	Boltzmann distribution (%)
1b-1		-1282.2608479	0.000000	44.58
1b-2		-1282.2608263	0.013554	43.57
1b-3		-1282.2588708	1.24063025	5.48
1b-4		-1282.2588154	1.27539375	5.17
1b-5		-1282.2574322	2.14335175	1.19

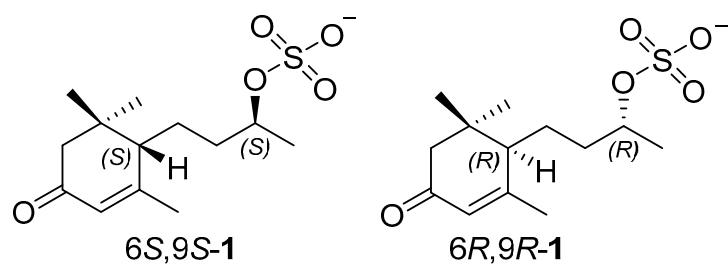


Figure S11. Two representative enantiomeric forms (*6S,9S*)-**1** and (*6R,9R*)-**1** used for conformation search based on molecular mechanics with MMFF force fields.

Blumenol C glucoside

Ground air-dried leaves of *Faramaea tamberlikiana* subsp. *sessifolia* (130 g) were exhaustively extracted with methanol at room temperature (3×2 d) yielding 4 g crude extract. Partitioning was performed as described in the main text for isolation of compound **1** and yielded 1.5 g *n*-butanol phase. This extract was subjected to CC over silica gel 60 (40–60 μ m particle size) eluted with mixtures of ethyl acetate and methanol. The fraction eluted with 20 % methanol (52.5 mg) was subjected to SEC and final purification by preparative TLC yielded 2.7 mg blumenol C glucoside.

White amorphous powder; MS, m/z : $[M+Na]^+$ 395.2038 (calcd for $[M+Na]^+$ 395.2040).

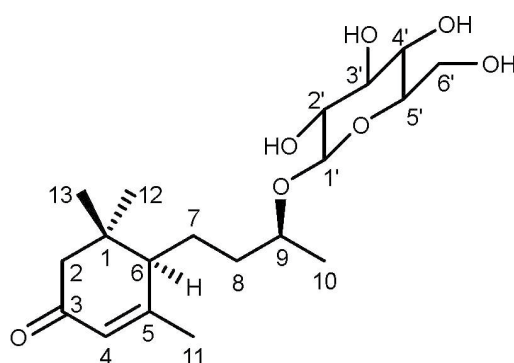


Figure S12. Structure of blumenol C glucoside.

Table S4. ^1H and ^{13}C NMR spectroscopic data for blumenol C glucoside in CD_3OD measured on a 600 MHz NMR. Numbering of carbon atoms is in accordance to Figure S12.

pos.	$\delta\ ^1\text{H}$ (ppm)	$\delta\ ^{13}\text{C}$ (ppm)
1	-	37.6, s
2	1.98 (d, 1H, 17.7), 2.48 (d, 1H, 17.7)	48.2, t
3	-	202.5, s
4	5.80 (m, 1H)	125.5, d
5	-	170.0, s
6	1.97 (m, 1H)	52.7, d
7	1.68 (m, 1H), 1.81 (m, 1H)	26.8, t
8	1.62 (m, 1H), 1.69 (m, 1H)	37.5, t
9	3.82 (m, 1H)	77.7, d
10	1.25 (d, 3H, 6.3)	22.0, q
11	2.04 (d, 3H, 1.3)	25.1, q
12	1.02 (s, 3H)	29.1, q
13	1.09 (s, 3H)	27.5, q
1'	4.31 (1H, d, 8.0)	104.1, d
2'	3.15 (1H, dd, 8.7, 8.0)	75.4, d
3'	3.33 (1H, dd, 8.7, 9.0)	78.4, d
4'	3.27 (1H, dd, 9.0, 8.4)	71.8, d
5'	3.25 (1H, m)	77.9, d
6'	3.65 (1H, dd, 12.3, 5.7) 3.85 (1H, dd, 12.2, 2.3)	62.9, t

Vomifoliol (= blumenol A)

Ground air-dried leaves of *Palicourea adusta* (124.5 g) were exhaustively extracted with methanol at room temperature (3×2 d) yielding 6.55 g crude extract which was partitioned as described in the main text for isolation of compound **1**. The chloroform, ethyl acetate and *n*-butanol phases were combined (650 mg) and subjected to repeated CC over silica gel 60 (0.2–0.5 mm particle size) with mixtures of petrol ether, ethyl acetate and methanol (46.1 mg) and 40–60 μm particle size with mixtures of chloroform and methanol. Final purification by preparative TLC afforded 2 mg vomifoliol also known as blumenol A.

White amorphous powder; MS, m/z : $[\text{M}+\text{Na}]^+$ 247.1302 (calcd for $[\text{M}+\text{Na}]^+$ 247.1304); Optical rotation: $[\alpha]_{\text{D}}^{20}$ (c 1.8 mg mL^{-1} , CH_3OH) = +93.9°.

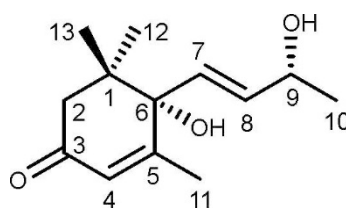


Figure S13. Structure of vomifoliol.

Table S5. ^1H and ^{13}C NMR of vomifoliol spectroscopic data in CD_3OD measured on a 600 MHz NMR. Numbering of carbon atoms is in accordance to Figure S13.

pos.	δ ^1H (ppm)	δ ^{13}C (ppm)
1	-	42.4, s
2	2.18 (d, 1H, 17.0), 2.50 (d, 1H, 17.0)	50.7, t
3	-	201.3, s
4	5.89 (m, 1H)	127.2, d
5	-	167.5, s
6	-	80.0, s
7	5.79 (d, 1H, 15.8),	130.0, d
8	5.82 (dd, 1H, 15.8, 5.1)	136.9, d
9	4.34 (dq, 1H, 5.1, 6.5)	68.7, d
10	1.26 (d, 3H, 6.5)	23.8, q
11	1.93 (d, 3H, 1.3)	19.6, q
12	1.06 (s, 3H)	23.4, q
13	1.03 (s, 3H)	24.5, q

Elemental analysis

Organic Elemental Micro-Analysis (EA) was originally developed by Fritz Pregl to characterize chemical substances and unveil their molecular composition [S1]. While the substance characterization is nowadays dominated by the subtle information gained by spectroscopic techniques characterization EA contributes complementary information to prove the purity of compounds. EA can also be applied to any material containing even a small fraction of organic substances. Service-requests related to carbon balance in environmental technology and biotechnology have become important applications of EA during the last decades.

The C/H/O-pattern of plant-derived material like the leaves of the investigated plants is generally dominated by the composition of cellulose ($C_6H_{10}O_5$ – 44.5 w-% C, 6.2 w-% H, 49.3 w-% O). The elements N and S are indicators of proteins and peptides as well as inorganic components (NH_4/NO_3 , SO_4).

Figures S14–S17 show the data in a semi-logarithmic plot to resolve the characteristics of low level signals (base line) and the maximum level data (CO_2 -peak) at the same time. There are four significant peaks assigned to nitrogen (N_2), carbon (CO_2), hydrogen (H_2O) and sulphur (SO_2). Numerical integration is done using linear base lines visible in the figures. A blank measurement is compensated by subtracting the trace prior to evaluation and plotting.

The diagrams include the evaluated raw data (area, A_X [V.s]), and the calibration factors f_X determined by a moving average model from calibration runs (third line). The blank file number is also indicated in the diagram. The analysis results w_X [w-%] and the sample weight m_0 [mg] are listed in the second line. Results are computed according to Equation S1.

$$w_X = \frac{f_X \cdot A_X}{m_0} \quad \text{Equ. S1}$$

In oxygen mode two peak areas are visible. The first sometimes splits into a part for hydrogen and nitrogen. Both cannot be used analytically. The third peak is assigned to oxygen (CO). The evaluation is done in the same way using Equation 1. Figures S14 and S15 show the raw data gained by analysis of AB 1 (elevated S-content) and AB 3 (normal S-content). Figures S16 and S17 represent examples from oxygen-determinations at the same two samples.

Ion analysis was done by capillary (free) zone electrophoresis using a CE 7100 system (Agilent). Separation was done at -30 kV along a 45 cm silica capillary with a 50 μ m bore. The separation was performed at pH 9.1 in a CHES-Arg buffer (50 mM cyclohexylaminen-ethane sulfonic acid, 20 mM L-arginine) containing 1 mM tetradecyl-ammonium hydroxide as EOF modifier. The ion profile was recorded using a TraceDec conductivity detector. Evaluation was done using a series of mixed anion-standards made from 0.1 M stock solutions prepared from high purity chemicals and MilliQ-water. An amount of 1.5 to 2.5 mg of the grinded sample material were extracted with MilliQ-water and analyzed after filtration with 0.4 μ m membrane filters.

Figure S18 shows the comparison of two samples with a 10 μM mixed anion standard containing bromide, chloride and sulfate. The analyte solutions contained about 2.6 mg of a sample in 50.0 mL water. Quantitative evaluation was based on peak areas. The calibration was based on a standard series from 5 to 100 $\mu\text{Mol L}^{-1}$. Figure S19 shows the calibration for chloride and sulfate.

Table S6. Detailed microchemical elemental analysis data for sample A–E.

Sample	Anal-No	w-%C	w-%H	w-%N	w-%S	w-%O	w-%Cl	w-%SO ₄	H:C	C:N	C:S	N:S	C:O	C:Cl	S in SO ₄	Sum	
A	210628/765	45,77	6,11	2,70	0,763				1,59	19,77	160,18	8,10					1,437 mg
	210628/766	45,77	6,11	2,69	0,744				1,59	19,85	164,27	8,28					1,308 mg
	210628/767	45,76	6,12	2,61	0,744				1,59	20,45	164,24	8,03					2,276 mg
	210714/148					41,72							1,46			97,02	
	210714/149					42,10							1,45			97,40	
	210714/150					40,93							1,49			96,23	
	210714/197					42,26							1,44			97,56	
	i210714/127						0,584	2,23							0,743		direct measured
	i210714/220						0,546	1,69							0,565		X93/739
	Average	45,77	6,11	2,67	0,750	41,75	0,565	1,96	1,59	20,02	162,87	8,14	1,46	239,10	0,654	97,61	X93/744
	σ	0,01	0,01	0,05	0,011	0,59	0,027	0,38									
B	210628/768	47,11	6,25	2,06	0,503				1,58	26,68	250,09	9,37					1,064 mg
	210628/769	48,38	6,47	2,15	0,371				1,59	26,25	348,22	13,27					1,826 mg
	210628/770	48,49	6,43	2,19	0,359				1,58	25,83	360,67	13,96					1,281 mg
	210714/151					39,63							1,61			96,55	
	210714/152					38,51							1,66			95,43	
	210714/153					39,56							1,62			96,48	
	210714/198					40,70							1,57			97,62	
	i210714/130						0,260	1,54							0,515		direct measured
	i210714/223						0,274	1,40							0,468		X93/740
	Average	47,99	6,38	2,13	0,411	39,60	0,267	1,47	1,58	26,24	311,81	11,88	1,61	530,57	0,491	96,79	X93/745
	σ	0,77	0,12	0,07	0,080	0,89	0,010	0,10									
C	210628/771	46,18	6,19	3,48	0,535				1,60	15,48	230,49	14,89					1,162 mg
	210628/772	46,07	6,24	3,33	0,532				1,61	16,14	231,24	14,33					2,019 mg
	210628/773	45,95	6,19	3,32	0,537				1,61	16,15	228,49	14,15					1,403 mg
	210714/154					40,08							1,53			96,26	
	210714/155					39,62							1,55			95,80	
	210714/156					40,04							1,53			96,22	
	210714/199					41,63							1,47			97,81	
	i210714/133						0,116	0,28							0,092		direct measured
	i210714/226						0,171	0,48							0,159		X93/741
	Average	46,07	6,21	3,38	0,535	40,34	0,144	0,38	1,61	15,91	230,07	14,46	1,52	947,56	0,125	96,67	X93/746
	σ	0,12	0,03	0,09	0,003	0,88	0,039	0,14									
D	210628/774	46,10	6,32	4,17	0,549				1,63	12,90	224,23	17,39					1,322 mg
	210628/775	46,24	6,34	4,13	0,545				1,63	13,06	226,56	17,35					1,676 mg
	210628/776	48,88	6,74	4,14	0,570				1,64	13,77	228,99	16,63					1,792 mg
	210714/157					38,61							1,62			96,85	
	210714/158					38,00							1,65			96,24	
	210714/159					38,90							1,61			97,14	
	210714/200					40,19							1,56			98,43	
	i210714/136						0,425	< 0,05									direct measured
	i210714/229						0,332	< 0,05									X93/742
	Average	47,07	6,47	4,15	0,555	38,93	0,379	< 0,05	1,64	13,24	226,62	17,11	1,61	367,10		97,54	X93/747
	σ	1,57	0,24	0,02	0,013	0,92	0,066										
E	210628/777	47,02	6,46	3,33	0,385				1,64	16,47	326,12	19,80					1,461 mg
	210628/778	47,20	6,41	3,37	0,386				1,62	16,34	326,52	19,98					1,326 mg
	210628/779	47,45	6,52	3,38	0,392				1,64	16,38	323,23	19,74					1,660 mg
	210714/160					39,66							1,59			97,09	
	210714/161					39,38							1,60			96,81	
	210714/162					39,29							1,60			96,72	
	i210714/139						0,321	< 0,05									X93/743
	i210714/232						0,306	< 0,05									X93/748
	Average	47,22	6,46	3,36	0,388	39,44	0,314	< 0,05	1,63	16,40	325,28	19,84	1,59	444,62		97,19	
	σ	0,22	0,06	0,03	0,004	0,19	0,011										
	theory															0,00	

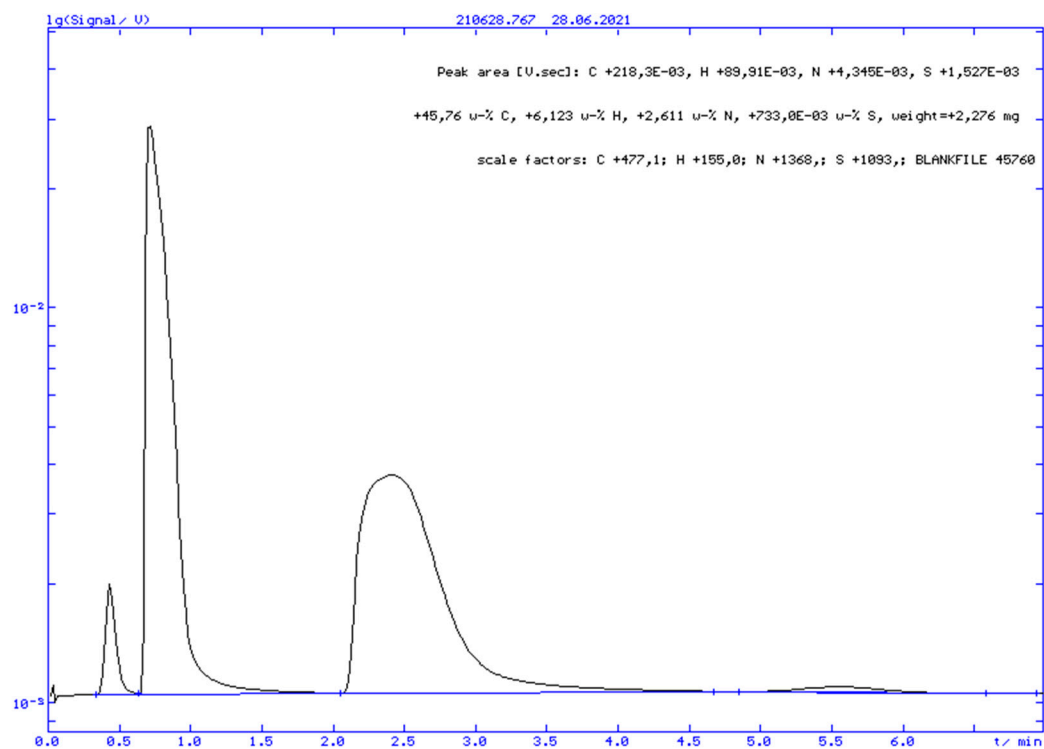


Figure S14. C/H/N/S-Analysis on EA 3000: TCD-trace of a C/H/N/S-run on sample A.

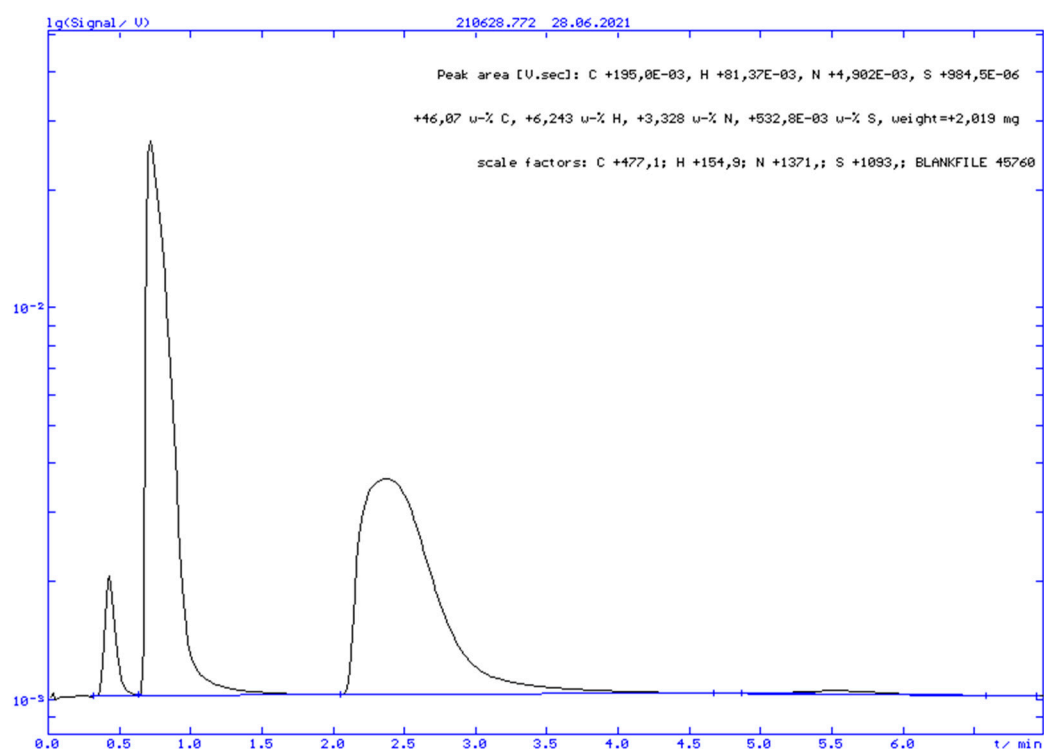


Figure S15. C/H/N/S-Analysis on EA3000: TCD-trace of a C/H/N/S-run on sample C.

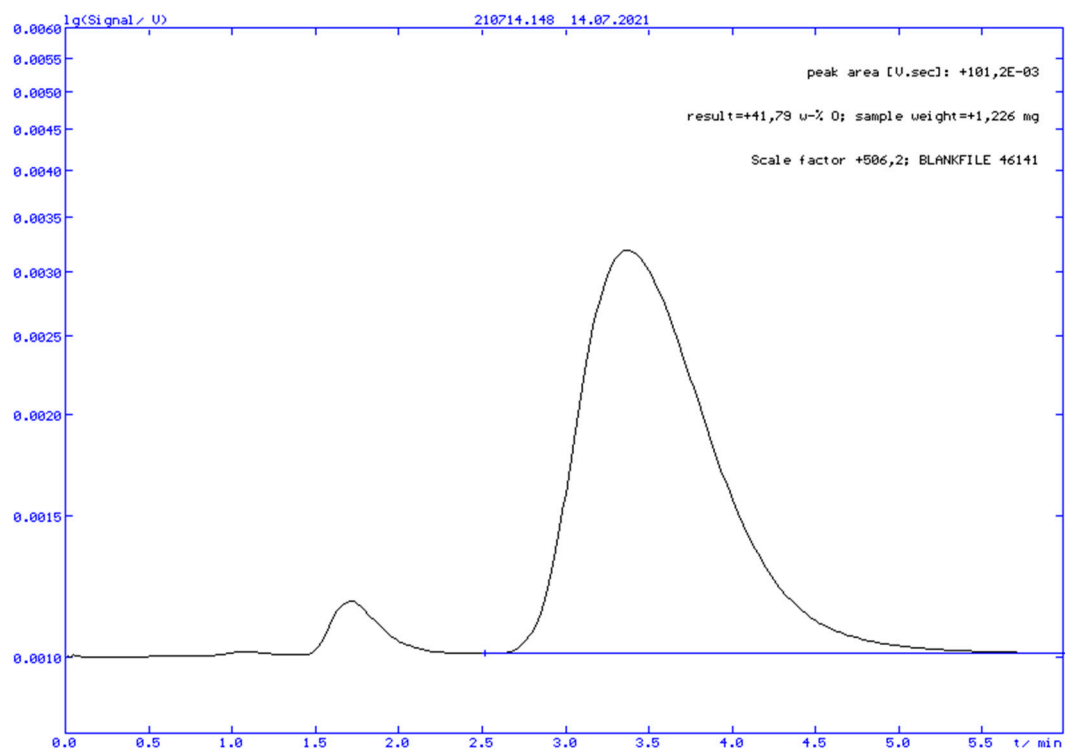


Figure S16. O-Analysis on EA3000 combined to the HT 1500 pyrolysis-system: TCD-trace of an analysis-run on sample A.

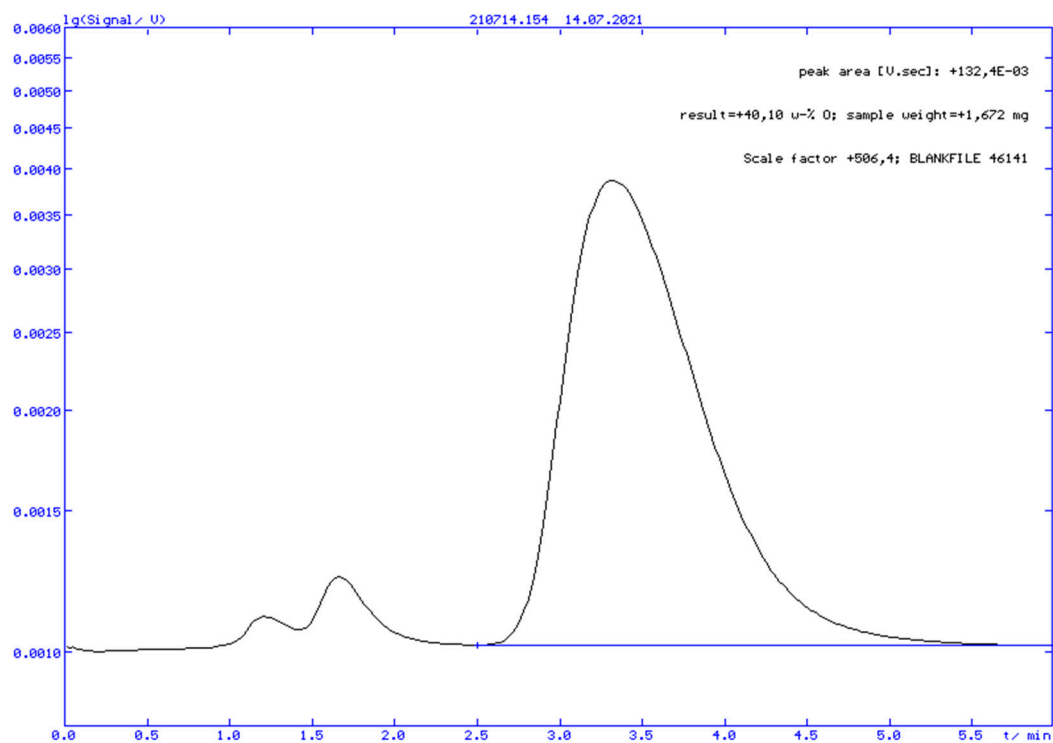


Figure S17. O-Analysis on EA3000 combined to the HT 1500 pyrolysis-system: TCD-trace of an analysis-run on sample C.

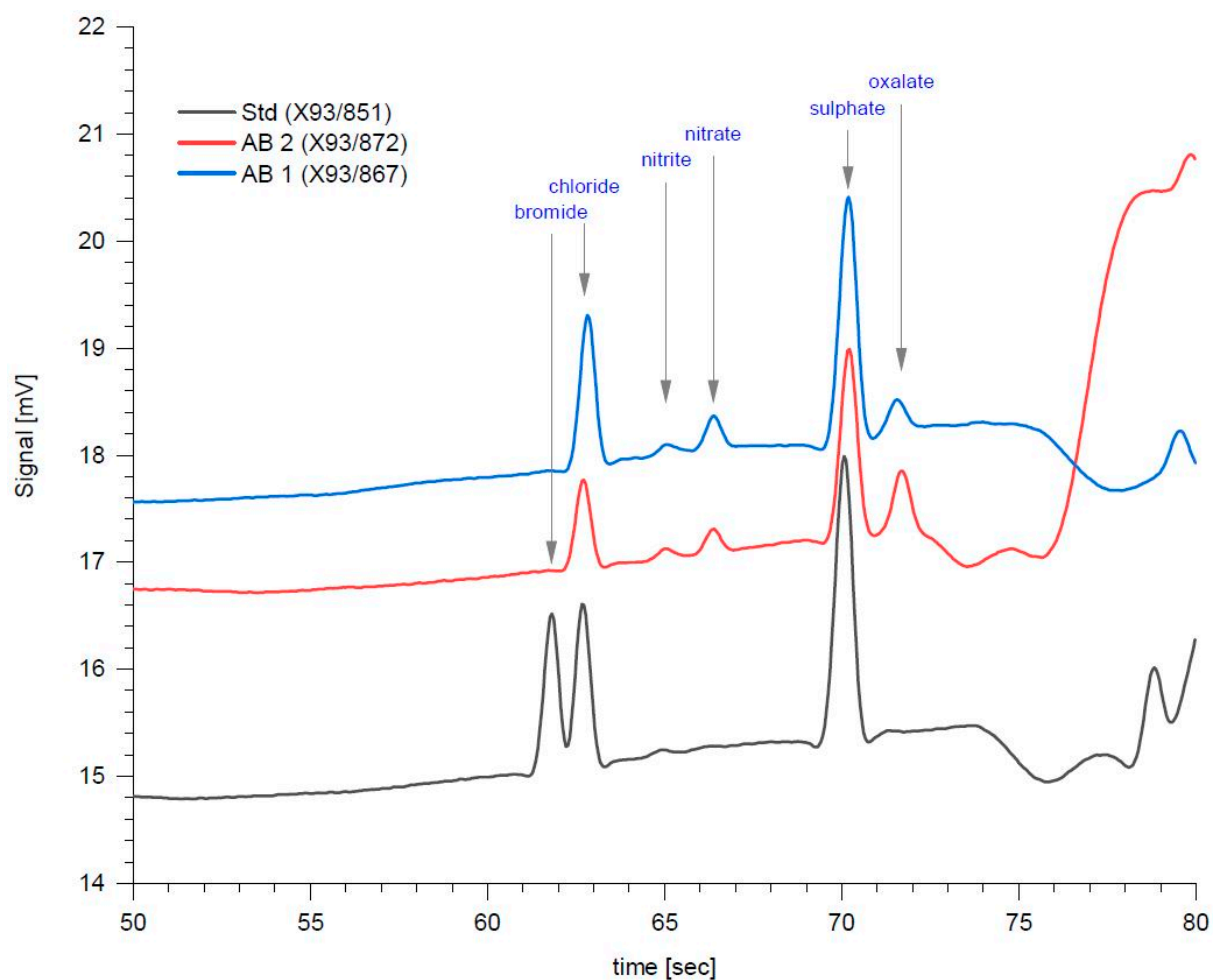


Figure S18. Comparison of two samples [A \equiv AB1; B \equiv AB2] with a 10 μ M mixed anion standard containing bromide, chloride and sulfate.

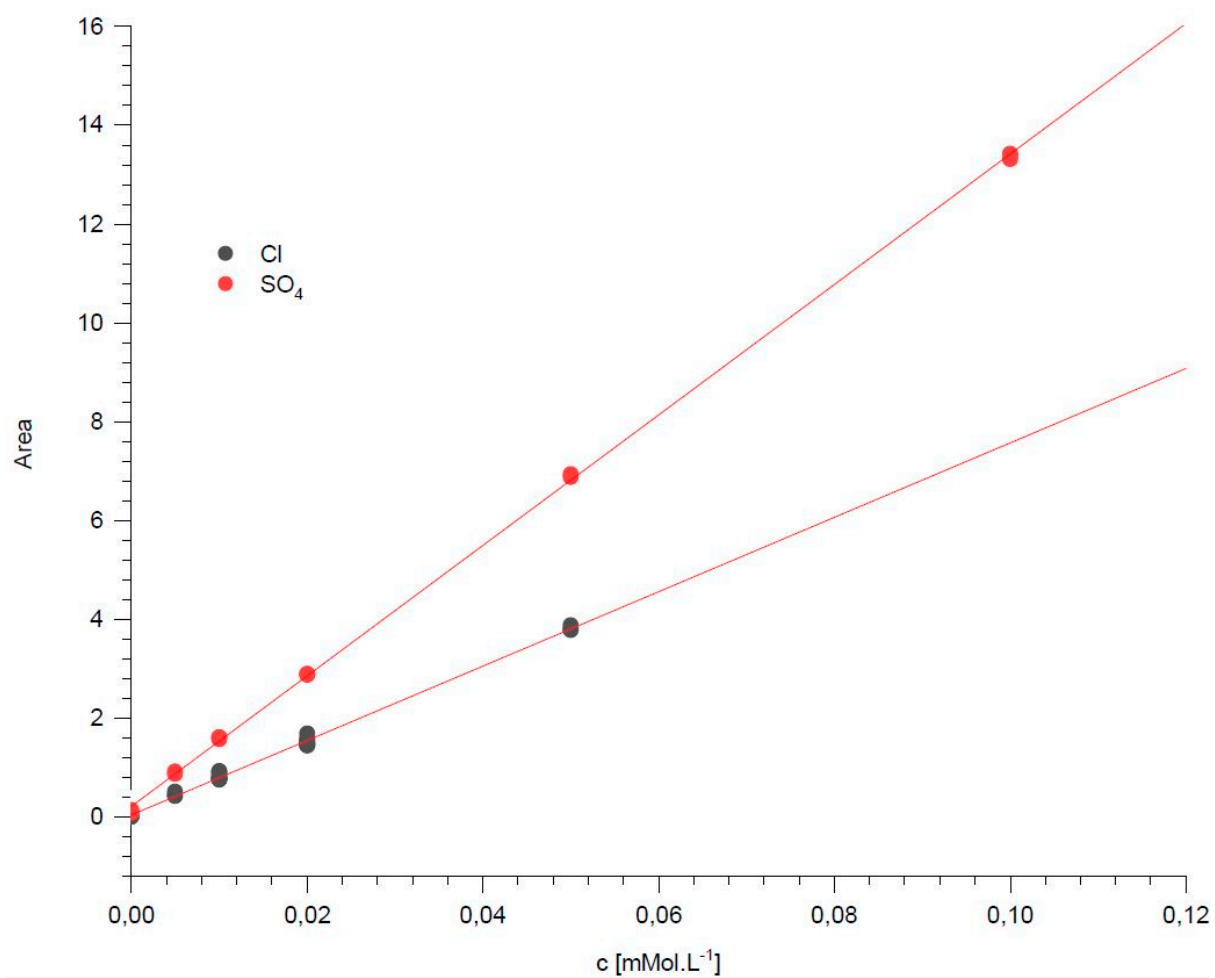


Figure S19. Calibration for chloride and sulfate.

References

- S1. Pregl, F. *Die Quantitative Organische Mikroanalyse*; Julius Springer-Verlag: Berlin, 1917.