

Supporting Information

Ultraperformance Liquid Chromatography Tandem Mass Spectrometry Determination of Cyanogenic Glucosides in *Trifolium* Species

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Figure S1. Structures of standards – linamarin and lotaustralin isolated from the aerial parts of *T. repens*

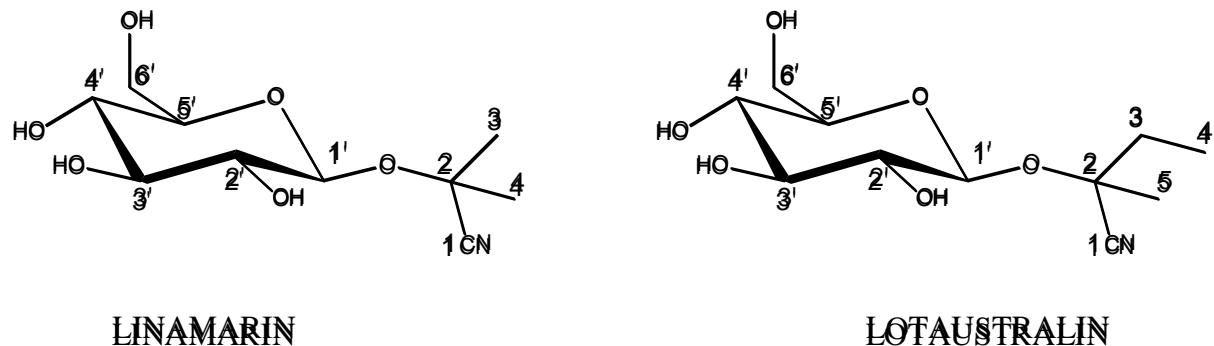
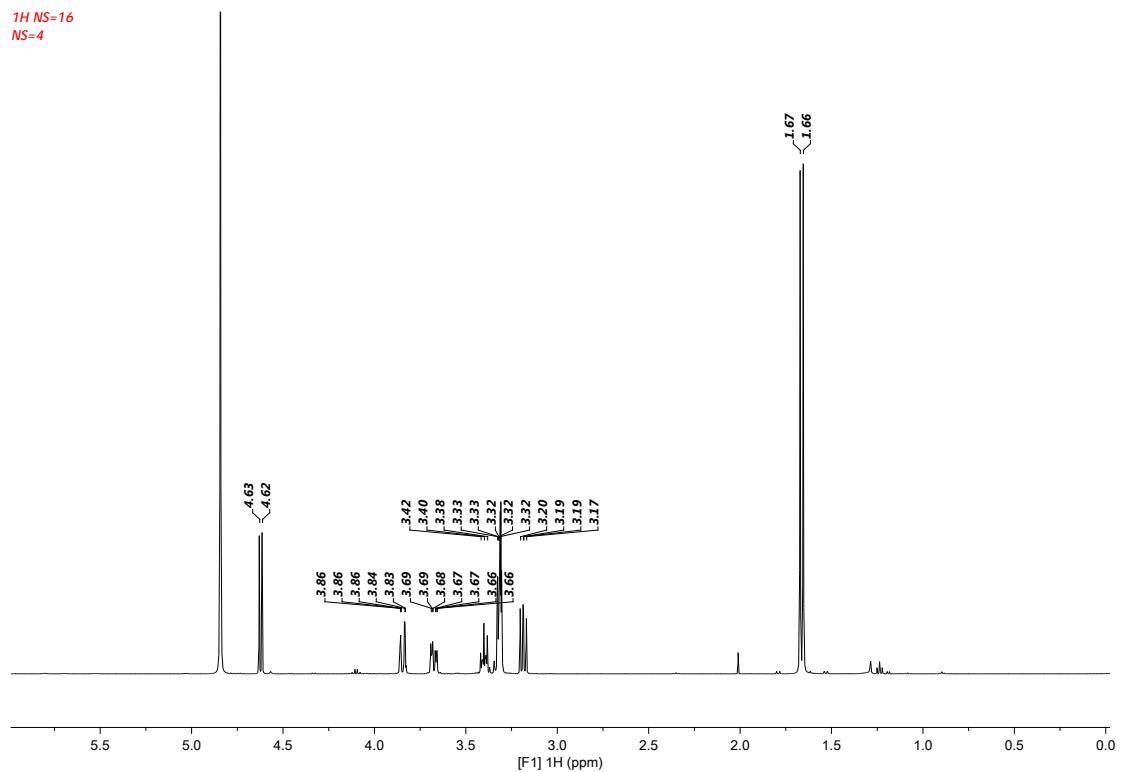


Table S1. NMR spectroscopic data (500 MHz) for linamarin and lotaustralin in MeOH-*d*₄

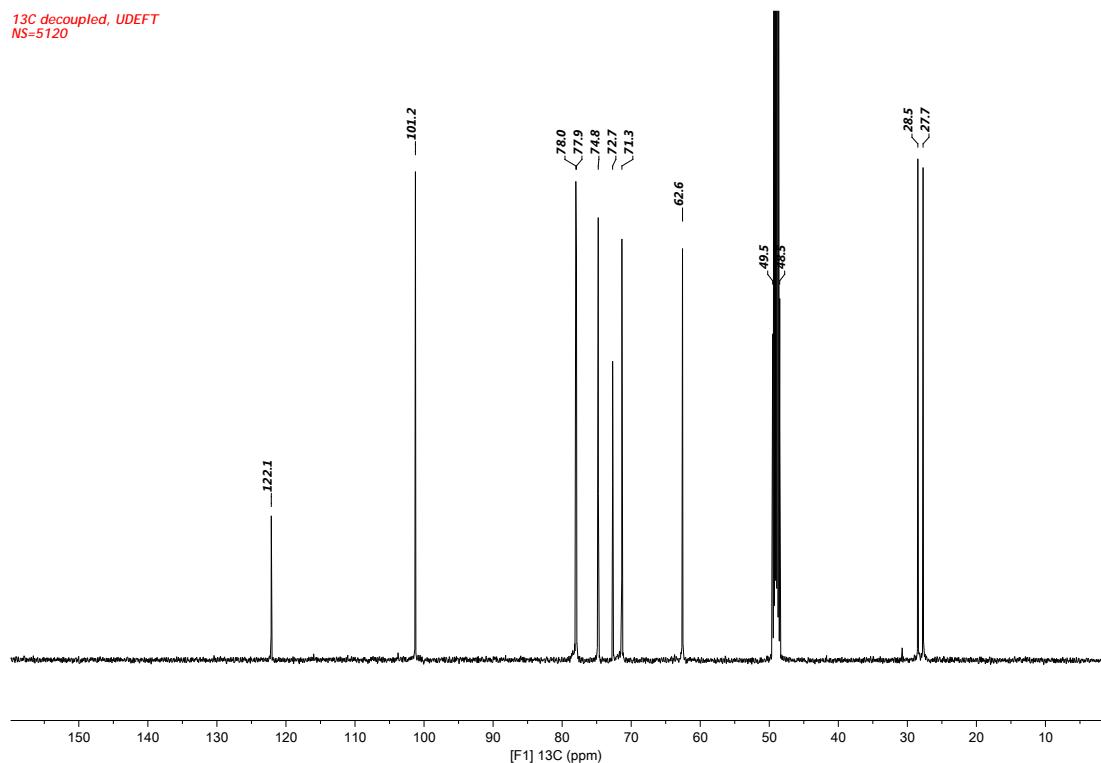
LINAMARIN			LOTAUSTRALIN		
Position	¹ H δ [ppm]	¹³ C δ [ppm]	Position	¹ H δ [ppm]	¹³ C δ [ppm]
1	-	122.1	1	-	121.7
2	-	72.7	2	-	76.3
3	1.66, s	27.7	3	1.97, dq (14.1, 7.4)	34.8
4	1.67, s			1.88, dq (14.1, 7.4)	
5	-	-	4	1.09, t (7.4)	8.8
β -Glucose					
1'	4.62, d (7.7)	101.2	1'	4.63, d (7.7)	100.8
2'	3.19, dd (9.1, 7.7)	74.8	2'	3.19, dd (8.9, 7.8)	74.8
3'	3.40, m	77.9	3'	3.40, t (8.5)	78.0
4'	3.32, m	71.3	4'	3.35, t (9.0)	71.3
5'	3.32, m	78.0	5'	3.31, m	78.0
6'	3.85, dd (11.9, 1.7)	62.6	6'	3.85, dd (11.9, 2.3)	62.6
	3.66, dd (12.0, 3.9)			3.69, dd (11.9, 5.1)	

Figure S2. ^1H and ^{13}C NMR spectra of linamarin and lotaustralin ($\text{MeOH}-d_4$, 500 MHz)

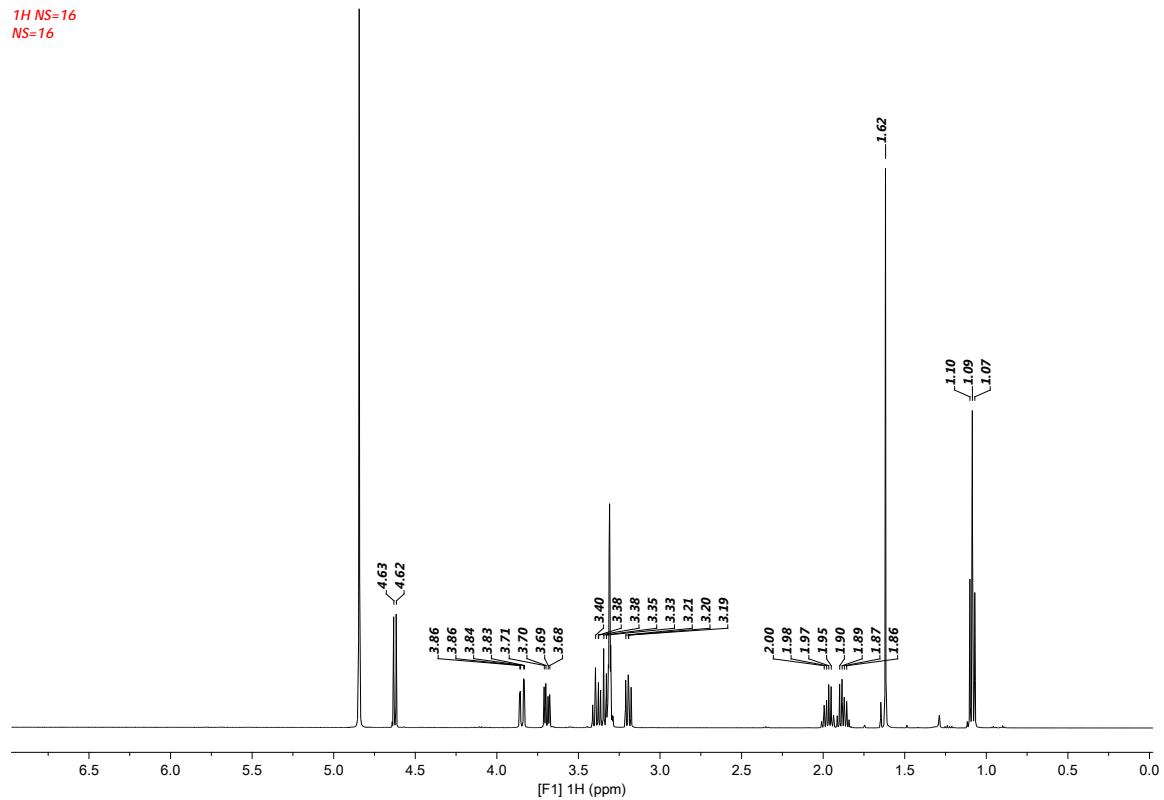
1. LINAMARIN – ^1H spectrum (500 MHz)



2. LINAMARIN – ^{13}C spectrum (125 MHz)



3. LOTAUSTRALIN – ^1H spectrum (500 MHz)



4. LOTAUSTRALIN – ^{13}C spectrum (125 MHz)

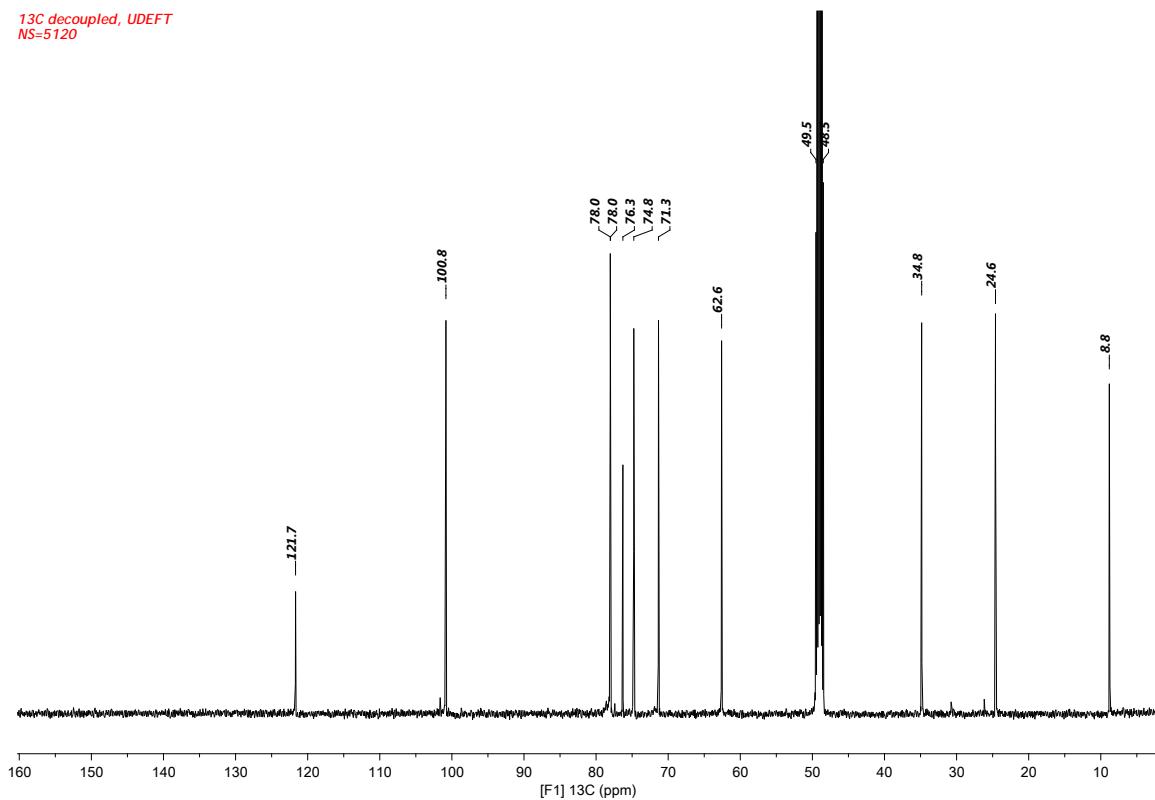


Figure S3. UPLC ESI-MS/MS ion chromatograms of linamarin (ESI-MS $[M+FA-H]^-$ at m/z 292, ESI-MS/MS at m/z 246 $[M-H]^-$, 160 $[M-85-H]^-$) and lotaustralin (ESI-MS $[M+FA-H]^-$ at m/z 306, ESI-MS/MS at m/z 260 $[M-H]^-$, 160 $[M-99-H]^-$) quantified in *Trifolium repens* var. *biasoletti* (Steud. & Hochst.) Asch. & Graebn., obtained with selected reaction monitoring (SRM).

