

New Methods Based on Tandem Mass Spectrometry to Rapidly and Completely Sequence Natural Product - Proanthocyanidins

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Overview

Purpose

Develop new and practicable methods to determine the sequence of heterogeneous A-type and B-type PAs in hops.

Method

- Solid phase extraction and reverse phase HPLC;
- On-Line HPLC-APCI-MS (MS);
- Off-Line LC-ESI-MSⁿ.

Result

The subunit sequences of hop PAs were identified by the diagnostic ions derived from HRF/RDA fission, HRF/BFF fission, RDA/HRF fission or QM fission combined with one of them, approaches reported here for the first time.

A valid ESI-MSⁿ database of hop PAs has been established. The fragmentation mechanisms were proposed based on the data obtained by ESI-MSⁿ.

MS fragmentations for direct analysis of PA mixtures extracted from hops were achieved without performing chromatographic separation thereby avoiding tedious work-up procedures.

Acknowledgement

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References

- (1) Li, H. J.; Deinzer, M. L. Structural Identification and Distribution of Proanthocyanidins in 13 Different Hops. J. Agric. Food Chem. 2006, 54, in press.

Introduction

Proanthocyanidins (PAs) are widely distributed throughout the plant kingdom, and are present as the second most abundant class of natural phenolic compounds after lignin. Hop PAs have received special attention in the beer brewing industry mainly because they contribute to haze formation, and stabilize the organoleptic properties and color of compounds that contribute to astringency and bitterness in the beer. The identification of these compounds is therefore crucial for studying their role in the brewing industry and for evaluating their possible human health effects.

The difficulty of extracting and purifying PAs, together with their instability and structural complexity, has made an understanding of the chemistry of this class of polyphenols fall well behind that of other constituents in hops. Moreover, quantities of PAs in hops are very limited, the estimated amount of total hop PAs ranges from 0.5 to 5% on a dry weight basis, depending on variety and geographic origin. For a long time, only a few hop PAs had been isolated and their structures determined[1], and reports for their systematic structural analysis were simply not available.

In addition, PA monomers and oligomers are easily changed during beer brewing, and the processing of some plants has been found to have a significant effect on the PA content. Therefore it is necessary to develop a method for rapidly identifying and distinguishing natural PAs.

Method

1. Crude PA mixture of hops was obtained through Sephadex LH-20 column.

2. Chromatographically Purified Individual Hop PAs were achieved by analytical 250 mmx 4.6 mm Synergi 4mm Hydro-RP-80A column up to 18 compounds.

3. On-Line HPLC-APCI-MS/MS was performed on a PE Sciex API III triple-quadrupole mass spectrometer in the positive ion MS mode and the source was equipped with a heated nebulizer interface kept at 480°C, argon-10% nitrogen as target gas at a thickness of ca. 1.9×10^{14} atoms per cm² using a collision energy of 20 V.

4. Off-Line LC-ESI-MSⁿ Mass spectrometry was recorded on Finnigan LCQ ion-trap mass spectrometer with an electrospray ionization (ESI) source in the positive mode: Spray voltage + 4.0 kV, flow rate ratio of sheath gas to auxiliary gas was 42/10, heated capillary temperature 170°C, the capillary voltage 17 V, tube lens offset 5 V, and scan range 120-2000.

Table 1. ESI Tandem Mass Product Ions (m/z), Retention Time (T_R) of Flavan-3-ol Monomers

Compn.	T _R (min)	[M-H] ⁺	HRF _C	RDA _C	BFF _C	BFF _C /H ₂ O	HRF _C /H ₂ O
1. C	25.31	291	165(126)	139(152)	169(122), 123	151(140), 123	147(144)
2. EC	22.32	291	165(126)	139(152)	169(122), 123	151(140), 123	147(144)
3. GC	17.19	307	181(126)	139(160)	169(130), 139	151(156), 139	163(144)

Table 2. Positive Ion ESI Tandem Mass Data (m/z), Retention Time (T_R) and Sequences of B-Type Dimers 4-10

Compn.	T _R (min)	[M-H] ⁺	QM ₁ ²⁺	QM ₂ ²⁺	HRF _C	RDA _C	RDA _C	RDA _C /H ₂ O/BFF _C	HRF _C /H ₂ O/BFF _C
4(E)AZ-(E)C	28.40	563	273	291	437(126)	427(136)	411(152)	285(152)	297(140)
5(E)GC-(E)C	15.55	595	305	—	469(126)	427(160)	443(152)	317(152)	329(140)
6(E)C-(E)GC	17.74	595	—	307	469(126)	443(152)	427(160)	301(160)	313(150)
7(E)C-(E)C	22.06	579	—	291	453(126)	427(152)	427(152)	301(152)	313(140)
8(E)C-(E)C	27.66	579	289	—	453(126)	427(152)	427(152)	301(152)	313(140)
9(E)C-(E)C	20.99	579	—	291	453(126)	427(152)	427(152)	301(152)	313(140)
10(E)C-(E)C	24.50	579	289	—	453(126)	427(152)	427(152)	301(152)	313(140)

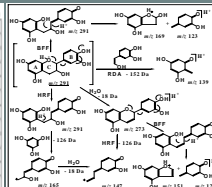
Table 3. Positive Ion ESI Tandem Mass Data (m/z), Retention Time (T_R) and Sequences of B-Type Trimers

Compn.	T _R (min)	[M-H] ⁺	QM ₁ ³⁺ /QM ₂ ³⁺	QM ₃ ³⁺	HRF _C ²⁺ /HRF _C ³⁺	RDA _C ²⁺ /RDA _C ³⁺	RDA _C ²⁺ /RDA _C ³⁺	H ₂ O/BFF _C
11(E)C-(E)GC-(E)C	10.25	883	—	593	595(283)443	757(126)	757(126)	757(126)
12(E)GC-(E)GC-(E)C	9.59	899	—	595	595(304)443	773(126)	773(126)	773(126)
13(E)C-(E)C-(E)C	19.60	867	—	579	579(283)427	741(126)	741(126)	741(126)
14(E)C-(E)C-(E)C	11.72	867	—	579	579(283)427	741(126)	741(126)	741(126)
15(E)C-(E)C-(E)C	28.50	867	—	579	579(283)427	741(126)	741(126)	741(126)

Table 4. Tandem Mass Diagnostic Ions (m/z) and Sequences of PA Oligomers and A-Type PAs

Compn.	Subunit Sequences	[M-H] ⁺	Diagnostic Ions
16	(E)C-(E)C-(E)C-(E)C	1137	447, 565, 579
17	(E)C-(E)C-(E)C-(E)C	1171	481, 599, 593
18	(E)C-(E)C-(E)C-(E)C	1187	497, 597, 593
19	(E)C-(E)C-(E)C-(E)C	1203	513, 619, 593
20	(E)C-(E)C-(E)C-(E)C	1401	1113, 1193, 867, 579
21	(E)C-(E)C-(E)C-(E)C	1419	1131, 1193, 867, 579
22	(E)C-(E)C-(E)C-(E)C	1477	1187, 1193, 867, 579
23	(E)C-(E)C-(E)C-(E)C	1701	1441, 1143, 1135, 867, 579
24	(E)C-A(E)C	593	441, 315
25	(E)C-A(E)C	593	441, 315
26	(E)C-A(E)C	601	501, 465, 383

Note: (E)AZ, (E)C and (E)GC are abbreviations for (epi)afzechin, (epi)catechin and (epi)gallocatechin, - A - represents an A-type interflavan linkage.



Note: RDA (retro-Diels-Alder Fission); HRF (heterocyclic ring fission); BFF (benzofuran-forming fission).

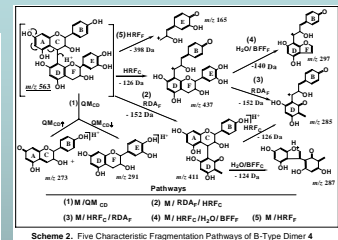
Results

The subunit sequences of hop PAs were identified by the diagnostic ions derived from HRF/RDA fission, HRF/BFF fission, RDA/HRF fission or QM fission combined with one of them, approaches that are reported here for the first time. All of the fragmentation mechanisms were proposed based on the data obtained by ESI-MSⁿ.

These new structural elucidation protocols enrich and expand the collection of analytical approaches available for sequencing PAs, and should be useful for the identification of other natural products. The new approach was found to provide for the rapid determination of hop PAs including those of the A-type in hop extracts, thereby allowing for their analyses without various tedious separation steps.

Because analytical separations are unnecessary, together with the exquisite sensitivity associated with mass spectrometry, makes this new analytical protocol based on LC/ESI-MSⁿ particularly useful for tracing the fate of proanthocyanidin oligomers during brewing as these compounds are extracted from hop cones directly into brew.

Furthermore, the proposed fragmentation mechanisms provide a strong theoretical basis on which an analytical method based on MRM (multiple reaction monitor) can be implemented to quantify PA monomers and oligomers in hops and beer, which has never been successful before.



Scheme 2. Five Characteristic Fragmentation Pathways of B-Type Dimer 4

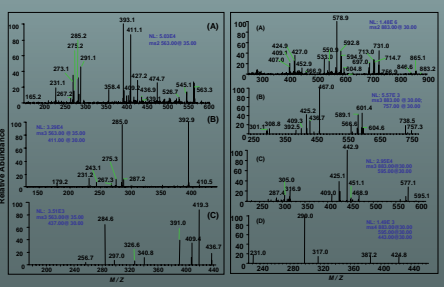


Figure 1. Positive Ion ESI Tandem Mass Spectra of B-Type Dimer 4

Figure 2. Positive Ion ESI Tandem Mass Spectra of B-Type Trimer 11

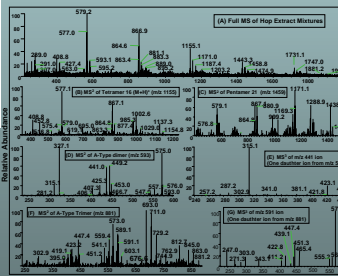


Figure 3. Positive Ion ESI Tandem Mass Spectra of PAs in Hop Extract Mixtures.

Conclusion

Newly established protocols using LC/ESI tandem mass spectrometry has been systematically exploited in this contribution to structurally elucidate and sequence 26 hop PAs ranging from monomers to pentamers. This is the first time to our knowledge that such an extent of diversity of the composition of PAs in hops has been observed by mass spectrometry.